EXHIBIT 4

3. Cellular Substrates of Brain Rhythms

Mircea Steriade

The rhythms of the electroencephalogram (EEG) are defined as regularly recurring waveforms of similar shape and duration. They have been recognized since the beginnings of EEG recordings in humans and animals and some of them have been thoroughly described during the 1930s and 1940s. However, the detailed mechanisms of EEG rhythms could be analyzed only during the past three decades and especially since 1980. This was due to the advent of modern methods allowing the description of electroresponsive properties and ionic conductances of various types of individual cells as well as the network operations that account for the collective oscillations of large neuronal populations. The aim of this chapter is to provide general notions about the cellular mechanisms of major EEG rhythms, with emphasis on the normal brain, and to frame these oscillations within the behavioral context of various states of vigilance.

The neuronal substrates of some EEG rhythms have begun to be elucidated up to their most intimate aspects, while other types of oscillations are far from being understood at the single-cell and population level. We now know in much detail the intrinsic neuronal properties and network synchronization of spindle oscillation (7–14 Hz), characterizing the state of light sleep, that are generated in the thalamus and whose widespread synchronization is determined by corticothalamic projections. We also know the cellular mechanisms underlying the recently described slow oscillation (less than 1 Hz) elaborated in the neocortex, reflected in the thalamus, and having the virtue of grouping other sleep rhythms (spindles and delta). The slow oscillation was originally described in intracellular recordings from anesthetized animals, and was subsequently recognized in EEG and single-unit extracellular recordings during natural sleep of animals and humans. We only begin to understand the sleep delta waves (1–4 Hz) at the level of single neurons and complex circuits, and we realize that the generic term *delta* comprises, in fact, a heterogeneous group of rhythms, with different mechanisms and levels of genesis, within the thalamus or neocortex. The theta rhythm (4–7 Hz), produced in the hippocampus and occurring during different forms of arousal, especially in rodents, was intensively studied at the cellular level, but its precise mechanisms are still subject to controversies. We have quite limited knowledge of various brain structures and neuronal types generating fast waves (20–60 Hz, mainly 30–40 Hz), so-called gamma oscillations, that appear in a sustained manner during highly aroused and attentive states as well as in the dreaming state of rapid-eye-movement (REM) sleep, but also, discontinuously, during slow-wave sleep. Finally, even though alpha waves, with frequencies largely overlapping those of spindling, have been described more than 60 years ago, we know little about the precise site of production and virtually nothing about the underlying neuronal mechanisms.

The order of sections in this chapter is not dictated by the frequencies of various rhythms, because waves ranging within a similar frequency range, such as alpha and spindling, are associated with quite opposite behavioral states (awareness and unconsciousness) and are probably generated at different brain levels (cerebral cortex and thalamus). Rather, I proceed from the synchronized EEG patterns of the sleepy thalamus and cerebral cortex (spindles, slow, and delta oscillations), and I describe the coalescence of these sleep rhythms within complex wave-sequences due to the corticothalamic volleys generated by the slow oscillation. Thus, although the description of the three distinct sleep oscillations is useful for didactic purposes, these rhythms are combined in the intact brain through reciprocal relations between the thalamus and cerebral cortex. I thereafter analyze the fast rhythms that accompany brain diffuse activation and focused attention, and discuss some modulatory systems that are essential for the shift from the closed brain during EEG-synchronized sleep to the open brain when information can be processed and analyzed. In sum, I will emphasize that the thalamus and cerebral cortex have to be considered as a unified oscillatory machine under the control of brainstem and forebrain modulatory systems.

Spindle Oscillation, Slow Oscillation, and K-complexes

Classically, spindles have been regarded as the epitome of EEG synchronization during the early stage of quiescent sleep. This type of oscillation is defined by the association of two distinct rhythms: waxing and waning spindle waves at 7–14 Hz within sequences lasting for 1–2 seconds, and the periodic recurrence of spindle sequences with a slow rhythm, generally 0.2 to 0.5 Hz (*top part* in Fig. 3.1). The slow rhythm of spindle sequences was only recently described (Steriade and Deschênes, 1984) but, with the benefit of hindsight, it can be detected in earlier recordings of humans and cat's bioelectrical activity. While the rhythm of 7–14 Hz was intensively investigated and its cellular bases are largely known, the knowledge of intrinsic currents or synaptic actions that are implicated in the slower rhythm of 0.2–0.5 Hz is only at its beginnings.

Spindles are generated within the thalamus, but their shape and long-range synchronization are decisively influenced by the cerebral cortex. That spindles are produced within the thalamus was first demonstrated by Morison and Bassett (1945) who showed that such waves are still recordable in the thalamus after total decortication and high brainstem transection. Thalamic spindles survive even more radical procedures, including the removal of the striatum and rhinencephalon (Villablanca, 1974). Although the intrinsic properties of

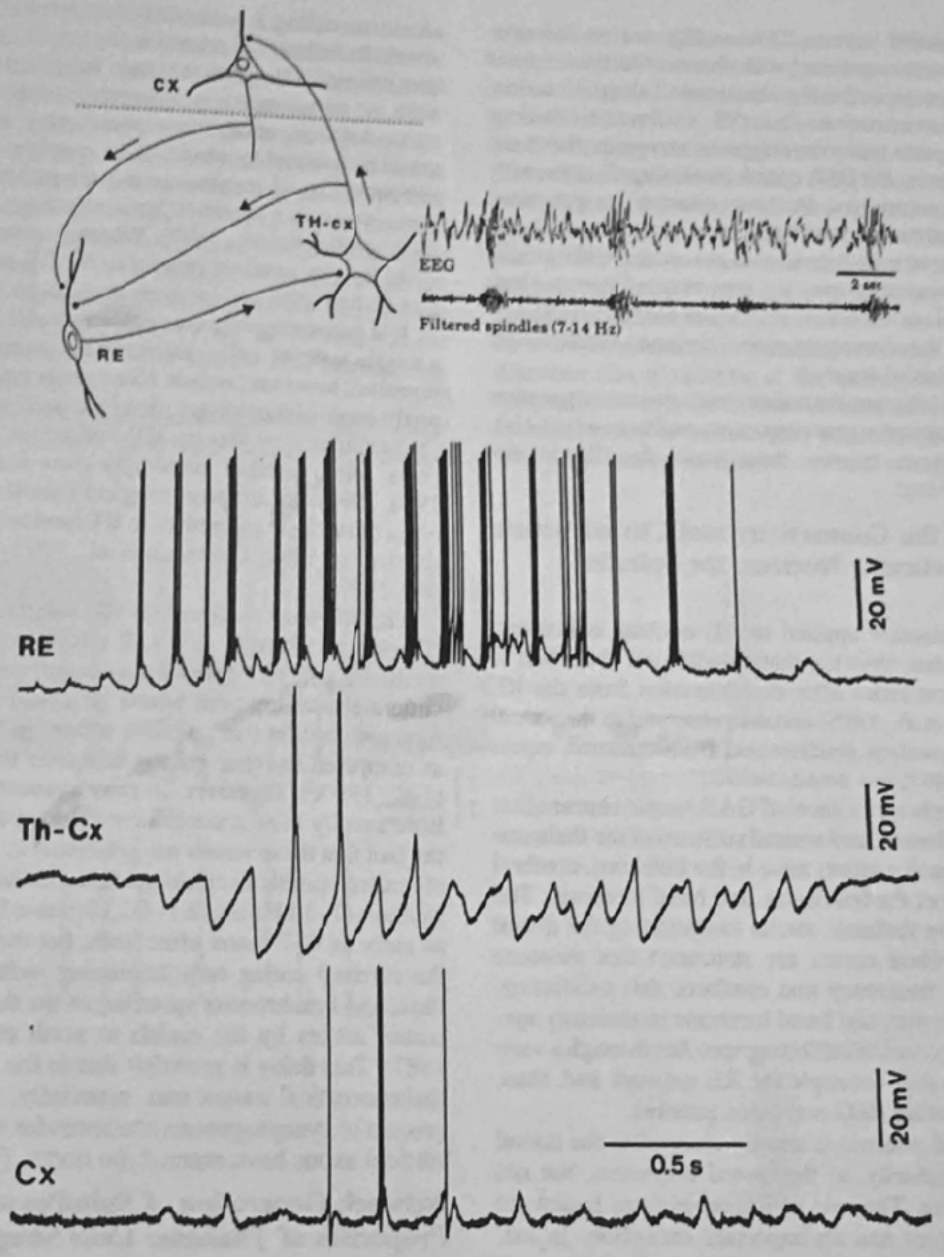

Figure 3.1. Spindle oscillations in reticular thalamic (*RE*), thalamocortical (*Th-Cx*, ventrolateral nucleus), and cortical (*Cx*, motor area) neurons. *Top:* Circuit of three neuronal types and two rhythms (7–14 Hz and 0.1–0.2 Hz) of spindle oscillations in cortical EEG. *Bottom:* Intracellular recordings in cats under barbiturate anesthesia. See text. (Modified from Steriade, M., and Deschênes, M. 1988. Intrathalamic and brainstem–thalamic networks involved in resting and alert states. In *Cellular Thalamic Mechanisms*, Eds. M. Bentivoglio and R. Spreafico, pp. 37–62. Amsterdam: Elsevier.)

individual thalamic cells play an important role in the patterning of sleep spindles (Steriade and Llinás, 1988), spindling is a network-generated oscillation in the recurrent inhibitory circuit including reticular (RE) thalamic cells, using gamma-aminobutyric acid (GABA) as transmitter, driven by thalamocortical (TC) neurons and projecting back to the latter (see *scheme* in Fig. 3.1).

Most of our knowledge of cellular bases of spindles derives from experimental studies in cat, the species of choice for the electrophysiological investigation of sleep spindles *in vivo*, and from *in vitro* studies in ferret's thalamic slices. Th sleep cycle is similarly organized in human and cats; EE spindles are similarly shaped in these species; and the R nucleus, that plays a cardinal role in the generation of spi dles, has similar ultrastructural features in cats and primat (see below). While cat's spindles are the electrographic lar mark for the transition from wakefulness to sleep and used as the main criterion for distinguishing the transit between these two behavioral states, in humans stage 1 sl is generally characterized by the change from alpha wa

to a mixed-frequency pattern of low-voltage waves, and spindles (in isolation or associated with sharp deflections termed *K-complexes*) appear in the so-called stage 2 sleep. However, stage 1 sleep may or may not coincide with perceived sleep onset in humans and many investigators recognize the clear-cut onset of sleep by the EEG correlates of stage 2, especially spindles and K-complexes. Because spindles are generated in the thalamus and may primarily appear in discrete foci, it is also possible that neuronal processes associated with spindle oscillations are operating from the very onset of human sleep but are not visible at the usual inspection at the cortical surface, because of the absence of global thalamic synchronization during this initial stage.

In this section I discuss the thalamically generated spindles (7–14 Hz), the cortically generated slow oscillation (<1 Hz), and the K-complexes, because these major sleep landmarks are intimately related.

Some Data on the Connectivity and Ultrastructure of Thalamic Reticular Nucleus, the Spindle Pacemaker

The term *pacemaker* applied to RE nucleus is justified by experimental data showing that spindles are abolished in dorsal thalamic territories after disconnection from the RE nucleus (Steriade et al., 1985) and are preserved in the rostral sector of the RE nucleus deafferented from thalamic inputs (Steriade et al., 1987; see details below).

The RE nucleus is a thin sheet of GABAergic neurons that covers the rostral, lateral, and ventral surfaces of the thalamus (Jones, 1985). Its major inputs arise in the thalamus, cerebral cortex, rostral part of the brainstem, and basal forebrain. The cortically projecting thalamic nuclei (constituting the dorsal thalamus) and cerebral cortex are structures that resonate within the spindle frequency and reinforce this oscillation. Distinctly, the brainstem and basal forebrain modulatory systems are mainly involved in inhibiting spindles through a variety of mechanisms that decouple the RE network and, thus, they assist in promoting EEG activation patterns.

The output of RE neurons is mostly directed to the dorsal thalamus and, subsidiarily, to the rostral brainstem, but not to the cerebral cortex. The rule of RE projections backward to the dorsal thalamus has an important exception. In cat, the anterior thalamic nuclei (interposed in the limbic circuit between the hippocampus and the cingular cortex) and the lateral habenular nucleus do not receive inputs from RE neurons (Steriade et al., 1984; Velayos et al., 1989). This connectional feature has a physiological counterpart: spindling is absent in anterior thalamic nuclei (Paré et al., 1987), in the projection areas of the cingular cortex (Leung and Borst, 1987), as well as in habenular neurons (Wilcox et al., 1988). All these structures, devoid of RE afferences, but being part of circuits comprising the septum, hippocampus, and entorhinal cortex, display theta rhythmicity that globally characterizes the limbic system.

The cells belonging to the RE nucleus are interconnected not only by means of their axons but also through their dendrites, which contain vesicles releasing GABA. The dendrodendritic contacts between RE neurons have been described in cats and primates (Deschênes et al., 1985; Yen et al., 1985; Asanuma, 1994) but have not been generally found in rodents. As will be described below, the significance of den dendritic linkages is related to the possibility of synchro tion of spindle oscillation within the pacemaking RE nuc with the consequence of synchronization of the whole t mus. Another, more economical way of synchroniz could be realized by electrotonic coupling of neurons. Im nohistochemical results suggest the presence of a gap j tion protein in a series of brain structures, including the nucleus (Nagy et al., 1988). Whether or not the immunor tivity for gap junction protein in the RE nucleus is relate glia or both glia and neurons remains to be determined

It is conventionally thought that the RE nucleus compr a single type of cells. Intracellular staining of RE neur revealed, however, two or three main types of RE elem on the basis of their shape, dendritic and axonal arborizati One cellular type has axonal collaterals within the RE cleus, while another seemingly does not (Spreafico et 1988, 1991). Electrophysiological data also indicate differ types of intrinsic properties in RE neurons (Llinás and Ge Barrientos, 1988; Contreras et al., 1992; Brunton and C pak, 1997).

The RE nucleus develops in ontogeny well before b (Altman and Bayer, 1979). If considering the bulk of d on the RE role as a spindle pacemaker (see below), this ea structural development would be conflicting with the co mon assumption that spindles appear quite late in ontoge as compared to other grapho-elements of the EEG (Steria et al., 1990c). However, in developmental studies, spindl have usually been recorded over the cortical surface, desp the fact that these waves are generated in the thalamus. Wh recording spindle oscillations in the thalamus of kittens, bo rhythms (7–14 Hz and 0.1–0.2 Hz) have been found to appe as early as 6–7 hours after birth, but they are transferred the cerebral cortex only beginning with the third to four days, and synchronous spindling in the thalamus and cerebr cortex occurs by the eighth to ninth days (Domich et a 1987). This delay is probably due to the late development thalamocortical axons and, especially, to the fact that th process of synaptogenesis continues for weeks after thalam cortical axons have entered the cortex (Wise et al., 1979).

Network Generation of Spindles and Intrinsic Properties of Thalamic Cells Shaping This Oscillation

An early model of spindle generation postulated the exis tence of a recurrent inhibitory circuit between TC cells and local-circuit inhibitory cells within the limits of dorsal thala mic nuclei, the latter being driven by intranuclear axonal col laterals of TC cells and imposing inhibitory postsynaptic po tentials (IPSPs) back onto TC cells that would fire postinhibitory rebound spike-bursts at the offset of IPSPs (Andersen and Andersson, 1968). In that model, which has the merit of emphasizing the propensity of TC cells to pro duce a powerful, intrinsic rebound discharge after a period of inhibition, the corticothalamic feedback played no role in spindling.

In subsequent studies it was shown that: (a) TC cells do not give rise to intranuclear axonal recurrent collaterals (Yen and Jones, 1983; Steriade and Deschênes, 1984); (b) local-circuit interneurons are not significantly implicated in spindle genesis as, after disconnection from RE nucleus, spindles are

abolished in thalamic nuclei possessing a great number of local interneurons and prolonged, rhythmic IPSPs in TC cells are transformed into short and arrythmic IPSPs; thus, *the spindle-related IPSPs in TC cells are produced by the other class of inhibitory thalamic cells, GABAergic RE neurons* (Steriade et al., 1985); (c) postinhibitory rebound spike-bursts in TC cells are transferred to cortex where they elicit rhythmic excitatory postsynaptic potentials (EPSPs) and occasional spike discharges in pyramidal neurons as well as other types of cortical cells (see Fig. 3.1); and (d) the cerebral cortex potentiates and synchronizes thalamic spindles (Steriade et al., 1972; Contreras et al., 1996a, 1997). These data and the recent developments from *in vivo* and *in vitro* experiments are further discussed below.

The idea that spindles are generated in the RE nucleus stemmed from the patterns of spindle sequences recorded intracellularly in RE and TC neurons. Whereas spindles develop in RE cells as a slowly growing and decaying depolarization with superimposed spike barrages within the frequency of 7–14 Hz, TC cells simultaneously display 7–14 Hz IPSPs that occasionally lead to high-frequency (200–400 Hz) rebound spike-bursts (Fig. 3.1). More recently, this result was obtained by means of dual simultaneous intracellular recordings from RE and TC cells *in vivo* (Timofeev and Steriade, 1996) and *in vitro* (Bal and McCormick, 1996). The spike-bursts of TC cells are stigmatic events during sleep spindles (Steriade and Llinás, 1988). These bursts represent an intrinsic property of thalamic cells, due to a special conductance that is inactive at the resting membrane potential (around −55 to −60 mV) or at more depolarized levels, but becomes ready to be activated when the membrane potential is hyperpolarized by about 7–15 mV, which is the case of TC

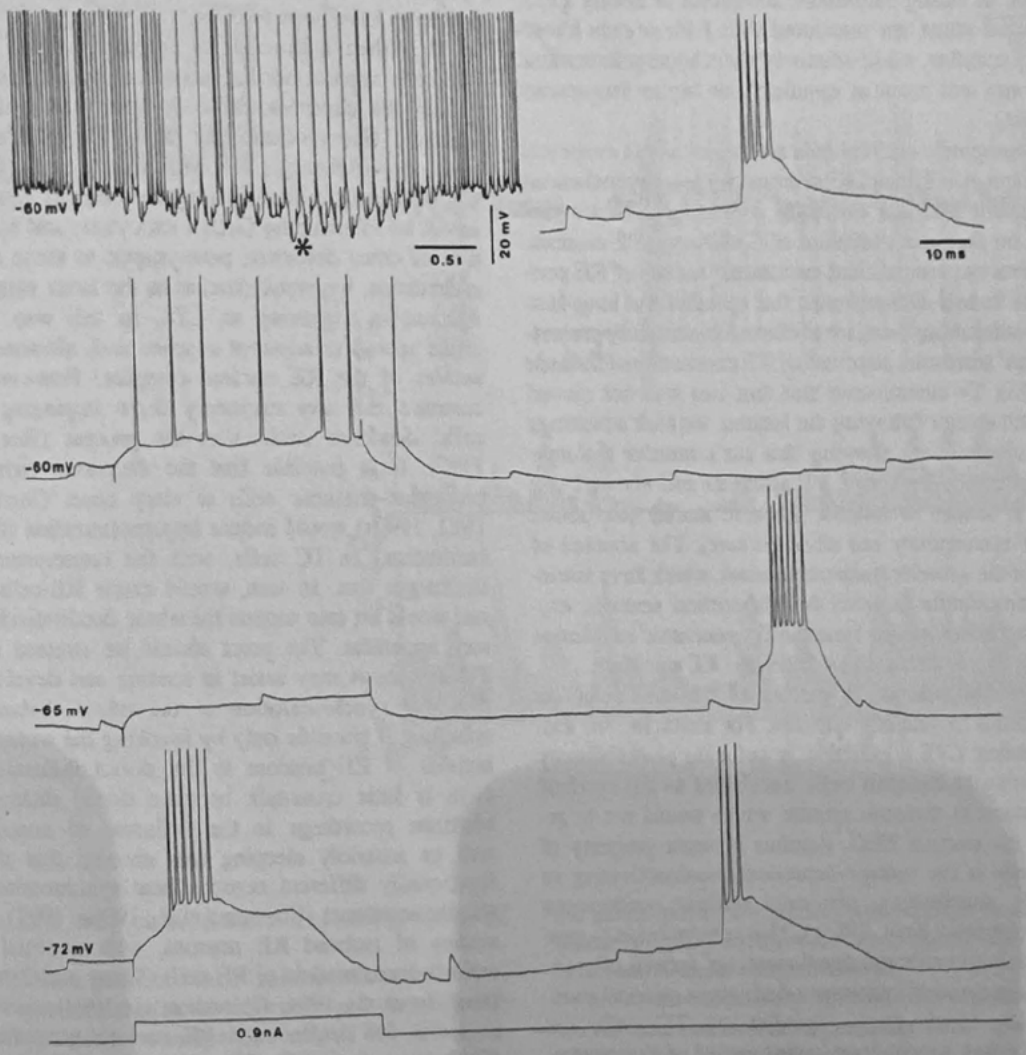

Figure 3.2. Two firing modes of thalamocortical cell. Intracellular recording in centrolateral intralaminar nucleus of cat under urethane anesthesia. *Top trace:* Tonic firing at resting membrane potential (V_m −60 mV) and rhythmic (~8 Hz) high-frequency spike bursts during episode with spontaneous hyperpolarization. Burst indicated by asterisk is expanded at right. *Bottom:* Responses to depolarizing current pulses (identical parameters) at different V_m by applying DC hyperpolarizing current. Note tonic firing at resting V_m (−60 mV), silent zone at −65 mV, and high-frequency burst at −72 mV. See also text. In this and similar figures, membrane potential is indicated at left. (From unpublished data.)

neurons during sleep with EEG synchronization (Fig. 3.2). As the high-frequency spike-bursts, consisting of fast sodium action potentials, are triggered by a slow spike that occurs much below the usual firing threshold, the latter is called low-threshold spike (LTS). The LTS was first discovered in inferior olivary neurons (Llinás and Yarom, 1981a, 1981b), thereafter was also found in thalamic cells (Deschênes et al., 1984; Jahnsen and Llinás, 1984a) and its calcium dependency was demonstrated *in vitro* (Jahnsen and Llinás, 1984b). The transient calcium conductance that underlies the LTS is located in the soma and/or proximal dendrites of TC cells (Jahnsen and Llinás, 1984; Zhou et al., 1997), whereas it is located in distal dendrites of RE neurons (Mulle et al., 1986; Huguenard and Prince, 1992; Destexhe et al., 1996).

The various frequencies of spindles, which have often been interpreted as reflecting different types of oscillations, do merely depend on various durations of the hyperpolarizations in TC neurons. Long duration (150–200 ms) hyperpolarizations, as during barbiturate anesthesia or deeply EEG-synchronized states, are associated with 7-Hz or even lower-frequency spindles, while relatively short hyperpolarizations (70–100 ms) will result in spindles with higher frequencies (10–14 Hz).

Since the spindle oscillation is associated with a reciprocal (inverse) image in RE and TC neurons, we have hypothesized that the cyclic IPSPs in cortically projecting cells are produced by the rhythmic excitation of GABAergic RE neurons. Transections experiments and excitotoxic lesions of RE perikarya have indeed demonstrated that spindles and long-lasting IPSPs subserving them are abolished in cortically projecting thalamic territories deprived of RE connections (Steriade et al., 1985). To demonstrate that this loss was not caused by traumatic events following the lesions, we took advantage of morphological data showing that cat's anterior thalamic cells are naturally devoid of RE afferents and showed that spindling is absent in anterior thalamic nuclei (see above section on connectivity and ultrastructure). The absence of spindling in the anterior thalamic neurons, which have intrinsic properties similar to other thalamocortical neurons, emphasizes that *spindling is a synaptically generated oscillation in a circuit that necessarily includes the RE nucleus.*

However, the intrinsic properties of thalamic cells are quite important in shaping spindles. For example, the calcium-dependent LTS is effective in inducing postinhibitory rebound bursts of thalamic cells transferred to the cerebral cortex. Without it, thalamic spindle waves would not be reflected on the cortical EEG. Another intrinsic property of thalamic cells is the voltage-dependent, noninactivating or very slowly inactivating, persistent sodium conductance $g_{Na(p)}$ (Jahnsen and Llinás, 1984b). This current helps to generate postinhibitory rebound depolarizations. Indeed, after intracellular injections of quaternary derivatives of local anesthetics which block $g_{Na(p)}$, spindles of TC cells are transformed into a single, long-lasting period of hyperpolarization (because it is unopposed by the persistent sodium current) and rhythmic rebounds within the frequency range of spindles disappear (Mulle et al., 1985). Finally, the calcium-dependent potassium conductance $g_{K(Ca)}$, or some voltage-dependent potassium currents, may prolong the long-lasting IPSPs generated in TC cells by RE neurons (Roy et al., 1984), and, thus, they assist in the production of the LTS and in inducing rebound bursts in TC cells.

The hypothesis that RE nucleus is the spindle pacemaker was finally strengthened by data showing that, after disconnection from its inputs (cerebral cortex, dorsal thalamus, and brainstem), the deafferented RE cells in the rostral pole of this nuclear complex continue to display both spindle rhythms (7–14 Hz and 0.2 Hz) and that spindle-related unit discharges are phase-locked with focal waves recorded by the same microelectrode (Steriade et al., 1987; Fig. 3.3). The spindle-related spike-bursts of the isolated RE nucleus are in all respects identical to those of the RE neurons in the brain-intact animals (Domich et al., 1986; Steriade et al., 1986, 1987). Then, two major experimental requirements to support the idea of a spindle pacemaker (abolition of the oscillation in target structures disconnected from the pacemaker, and preservation of oscillation in the isolated rostral pole of RE nucleus) have been fulfilled.

It is then reasonable to propose that the deafferented RE cells support oscillations through an avalanche process within the dendrodendritic synaptic junctions of the RE nucleus. Hyperpolarization through dendrodendritic synapses of GABAergic RE cells would produce an LTS in the postsynaptic element (say *a*); calcium entry in neuron *a* will be followed by GABA exocytosis and hyperpolarization of other dendrites, postsynaptic to those of the cell's *a* dendrites; hyperpolarization in the latter elements would succeed in triggering an LTS. In this way, oscillations could spread to adjacent neurons and, ultimately, to large sectors of the RE nuclear complex. However, we have assumed that any excitatory drive impinging upon RE-cells' dendrites could start the process (Steriade et al., 1987). It is possible that the decreased firing rates of brainstem–thalamic cells at sleep onset (Steriade et al., 1982, 1990a) would induce hyperpolarization (through disfacilitation) in TC cells, with the consequence of burst discharges that, in turn, would excite RE-cells' dendrites and would set into motion the whole dendrodendritic inhibitory apparatus. The point should be stressed that, while TC–RE loops may assist in starting and developing spindles, the synchronization of the whole thalamus during spindling is possible only by invoking the widespread projections of RE neurons to the dorsal thalamus, because there is little cross-talk between dorsal thalamic nuclei. Multisite recordings in the thalamus of anesthetized as well as naturally sleeping cats showed that distant and functionally different neurons beat synchronously during spindle sequences (Contreras et al., 1996a, 1997). Modeling studies of isolated RE neurons, with minimal or more realistic ionic models of RE cells (Wang and Rinzel, 1993; Destexhe et al., 1994; Golomb et al., 1994), confirmed the idea that the deafferented RE nucleus generate spindles (Steriade et al., 1987). The modeling studies reached the conclusion that densely interconnected RE cells with $GABA_A$ or $GABA_B$ synapses are capable of spindle oscillations and that a modest excitation from input sources is effective in fully synchronizing the isolated RE network.

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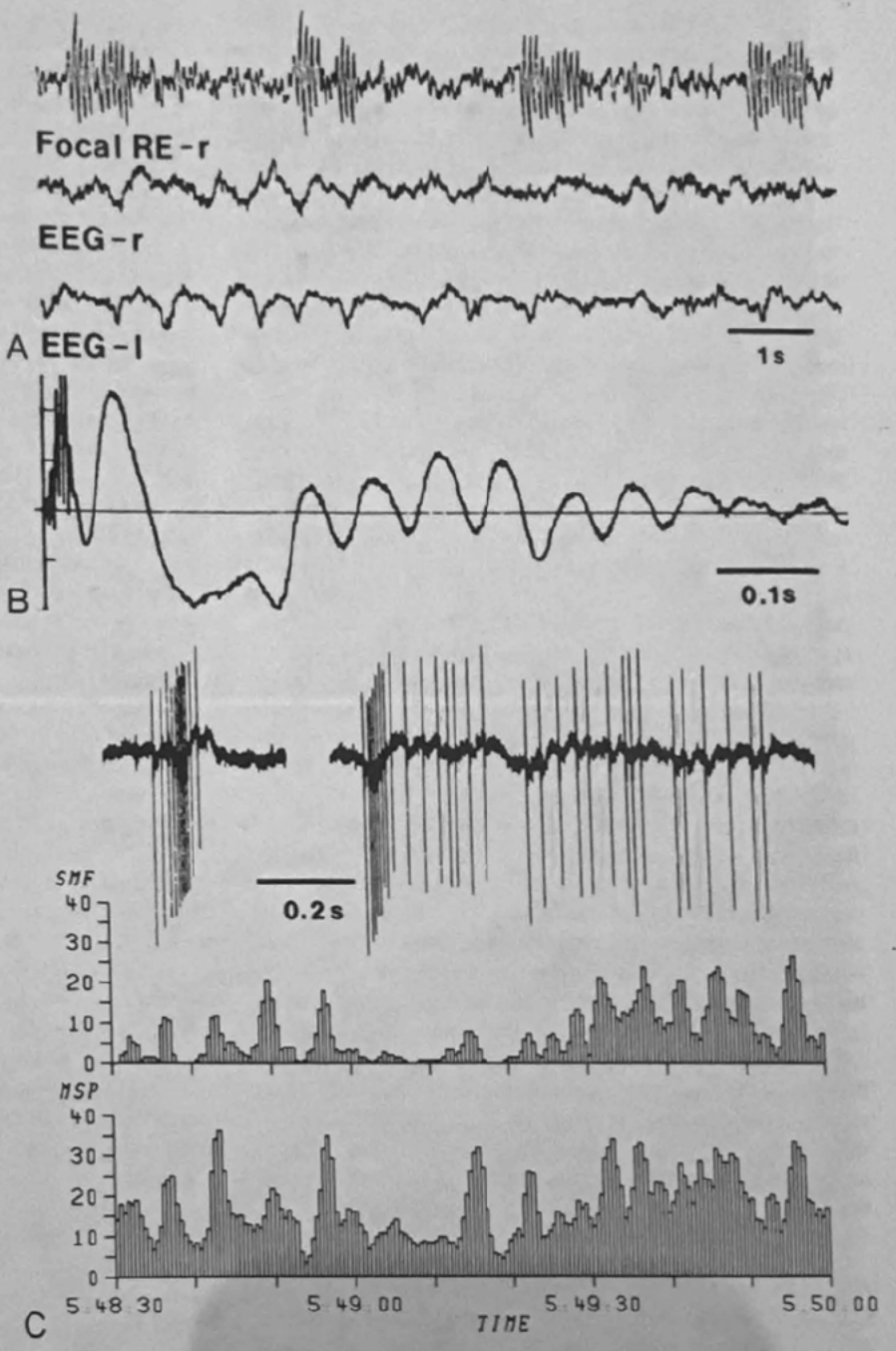


Figure 3.3. The deafferented reticular thalamic (*RE*) nucleus of cat generates spindle rhythmicity. For histology of transections that created an isolated island containing the rostral pole of the RE nucleus, see Figures 1 and 2 in Steriade et al. (1987). **A.** Normal cyclic recurrence of spindle sequences in the rostral pole of the RE nucleus recorded by means of a microelectrode; absence of spindle rhythms (but persistence of delta waves) in cortical EEG recordings following thalamic transections. **B.** Oscillations within spindle frequency evoked in the rostral pole of the RE nucleus by stimulating (five-shock train) the white matter overlying the caudate nucleus (50 averaged traces). **C.** Slow rhythm of spindle sequences and related cell burst oscillations in the rostral pole of the RE nucleus deafferented by thalamic and corona radiata transections. Discharges of a single RE neuron were simultaneously recorded with focal spindle oscillations by the same microelectrode. Sequential mean frequency (*SMF*) of the neuron is depicted with the normalized amplitudes of focal waves filtered from spindle waves (*MSP*). Abscissa indicates real time. *Top:* Two (short and long) bursts from the same period. (Modified from Steriade, M., Domich, L., Oakson, G., and Deschênes, M. 1987. The deafferented reticular thalamic nucleus generates spindle rhythmicity. J. Neurophysiol. 57:260–273).

The excitation may come from cortex and/or from TC cells.

Investigations in ferret visual thalamic slices maintained *in vitro*, containing the lateral geniculate and perigeniculate (caudal RE) nuclei, have analyzed the role of TC–RE interactions in spindle genesis and have specified the various receptors involved in the TC-to-RE excitation as well as in the GABA-mediated IPSPs imposed by RE onto TC neurons (von Krosigk et al., 1993; Bal et al., 1995a,b). The absence of spindles in the perigeniculate nucleus disconnected from the lateral geniculate nucleus (von Krosigk et al., 1993) may be explained by different factors in slices, such as (a) a less intact and complete collection of RE cells; and (b) the absence of brainstem modulatory systems with depolarizing actions on RE neurons. Indeed, a modeling study (Destexhe et al., 1994b) has shown that network of isolated RE cells do not display spindle oscillatory behavior when no noradrenergic(NA)/serotonergic(5-HT) synapses are activated, but RE cells were brought to oscillation by activating 20% of the depolarizing NA/5-HT synapses.

The role of corticothalamic feedback in shaping, generation, and synchronization of spindles was demonstrated by: (a) different shape and duration of spindles as a function of cortical stimulation (Contreras and Steriade, 1996) or by comparing thalamic spindles in intact-cortex and decorticated animals (Timofeev and Steriade, 1996); corticothalamic volleys produce brief and waning spindle sequences that lack the initial waxing component because these volleys succeed in entraining, right from the start, a great or the totality of thalamic cellular population implicated in spindle genesis; (b) eliciting spindles as response to cortical stimulation, even after contralateral cortical volleys to avoid antidromic activation of TC axons and collateral excitation of RE neurons (Steriade et al., 1972); (c) synchronizing RE cells, within spindle frequency, in response to cortical stimulation (Contreras and Steriade, 1996); and (d) synchronizing widespread thalamic territories to produce nearly simultaneous spindle sequences, as after decortication the coherence of spindles is much less organized (Contreras et al., 1996a, 1997) (Fig. 3.4). The propagation of spindles in thalamic slices (Kim et al., 1995) is probably due, at least partially, to the absence of corticothalamic projections and reduced background synaptic activity. The role of the cortical slow oscillation in grouping and synchronizing spindles is discussed below.

As TC neurons spend much of their sleep time during spindle-related IPSPs, there is a powerful inhibition of incoming messages in their route to the cerebral cortex. Recording field potentials evoked by stimulation of prethalamic axons (a method that permits the monitoring of the presynaptic deflection reflecting the magnitude of the afferent volley, together with the synaptically relayed, thalamically generated waves) revealed that the thalamus is the first station where afferent signals are completely blocked from the very onset of sleep. This obliteration of synaptic transmission in the thalamus leads to the deafferentation of the cerebral cortex, a prerequisite for the process of falling asleep (Steriade et al., 1969; Steriade, 1984). More recently, intracellular recordings from thalamic and cortical neurons have shown that, because of their hyperpolarization during sleep, TC cells do not transfer to cortex signals from prethalamic relay station, whereas the internal (corticocortical and corticothalamic) dialogue of the brain may be maintained during sleep (Timofeev et al., 1996).

Incremental Thalamocortical Responses within Spindle Frequencies: Augmenting and Recruiting Waves

Repetitive (7–14 Hz) stimulation of dorsal thalamic nuclei evokes responses in projection cortical areas that increase in size during the pulse-train (Fig. 3.5A). This is the common way to mimic spindles in order to study the mechanisms of their waxing and waning pattern. Two types of incremental responses are known since their original description by Morison and Dempsey (1942): positive–negative waves at the cortical surface, also called *augmenting* response; and initially surface-negative waves, also termed *recruiting* responses.

The different polarities of these two types of incremental responses reflect different projections of various thalamic nuclei to cortical layers. Most so-called "specific" dorsal thalamic nuclei preferentially project to midlayers III–IV of cortical areas, while some thalamic (for example, ventromedial and centrolateral) nuclei have prevalent projections to the superficial layer I. The surface-positivity (depth-negativity) of the augmenting response results from excitatory thalamocortical inputs, which create sinks in layers IV and supervening part of layer III, with current flow along the vertical conductors represented by the apical dendrites of deeply lying, pyramidal-shaped neurons. The surface-negative (depth-positive) wave of recruiting responses results from direct depolarization of apical dendrites in layer I where recruiting-eliciting thalamic nuclei project.

As to the negative component that follows the initially positive deflection in augmenting responses, it tends to be marked over wider cortical areas than the positive wave. This aspect is due to the fact that a series of "specific" thalamic nuclei, in addition to their prevalent projections to midlayers III–IV of restricted cortical regions, also project quite widely to layer I. The complexity of thalamocortical systems is such that a single cortical area receives afferents from multiple thalamic nuclei. This is the case, for example, of the cortical associational area 5, toward which the lateral posterior thalamic nucleus projects to layers III–IV and the ventral anterior thalamic nucleus projects to layer I. The result is an augmenting-type of response in cortical area 5 by stimulating lateral posterior nucleus, and a recruiting-type of response in the same cortical area by stimulating the ventral anterior nucleus (Steriade, 1978) (Fig. 3.5B).

The processes accounting for the development of thalamocortical incremental responses are an increased amplitude of the secondary depolarizing component and an attenuation of hyperpolarizing potentials (Purpura et al., 1964; Creutzfeldt et al., 1966). The augmented secondary excitation in cortical neurons takes the form of burst discharges, and it occurs in conjunction with a decrease in the primary single-spike excitation (Steriade, 1978) (Fig. 3.5A). The precise mechanisms of the increased secondary depolarization in cortical neurons are not known, although some possibilities (activation of *N*-methyl-D-aspartate, NMDA, or metabotropic glutamate receptors) are open and should be investigated. The augmented secondary depolarizing component is quite sensitive to changes in the state of vigilance: it is selectively reduced or abolished by midbrain reticular stimulation and during naturally brain-activated states, such as waking and rapid eye movement (REM) sleep (Steriade, 1970, 1981).

Are augmenting and recruiting thalamocortical responses primarily due to events generated at the thalamic stimulated site (such as inhibitory potentials with subsequent rebound excitations transferred to the cerebral cortex), or do they reflect cortical events? That the cerebral cortex has the circuitry to elaborate such incremental responses in the absence of the thalamus was demonstrated by evoking augmenting responses to stimulation of white matter after lesions of the appropriate thalamic nuclei (Morin and Steriade, 1981) or by antidromic activation of corticothalamic axons (Ferster and Lindström, 1986). The spatiotemporal features of augmenting responses have been investigated in motor cortex and it was proposed that the initiation of these responses depends on the intrinsic properties and synaptic interconnections of layer V pyramidal cells (Cas

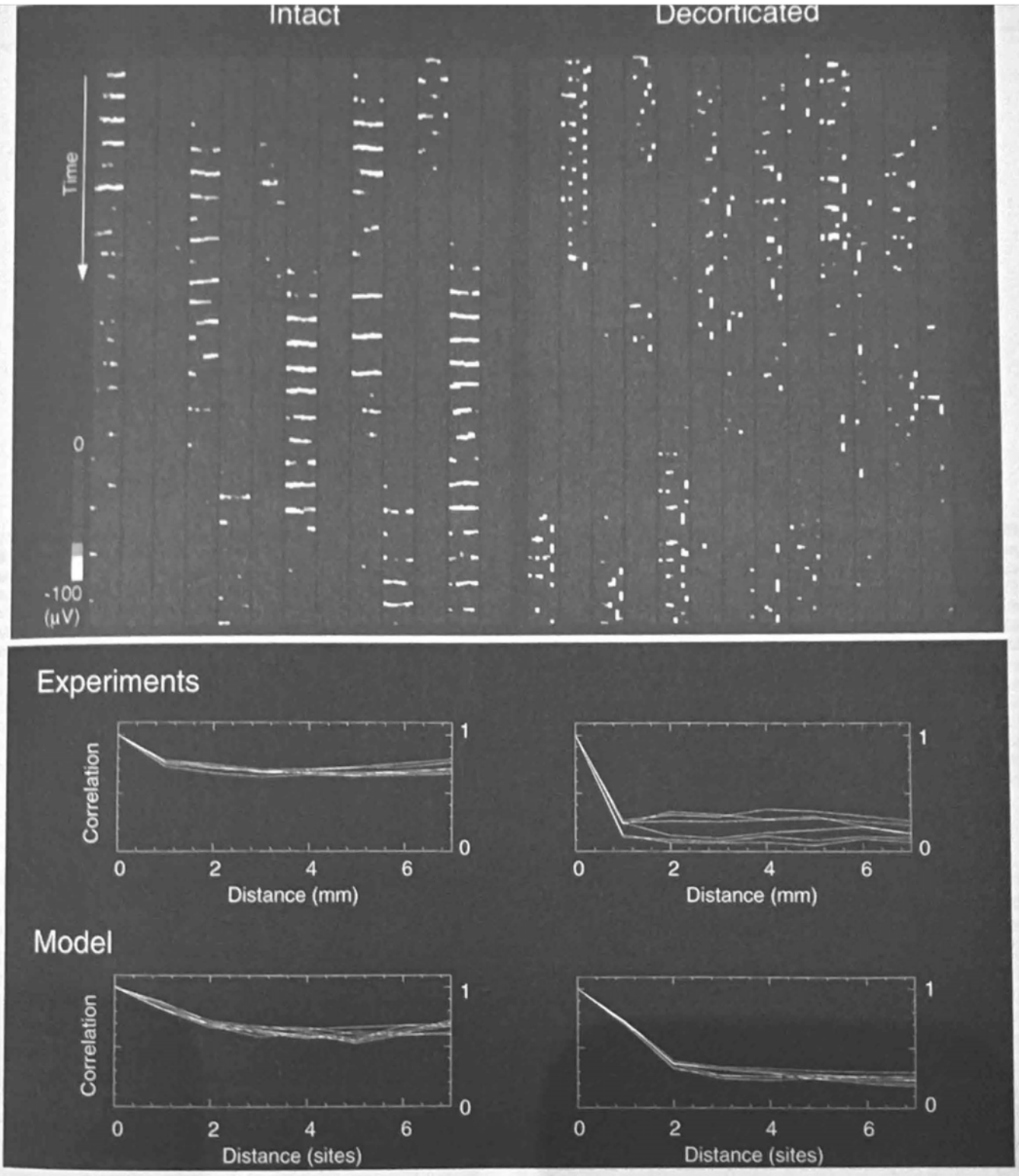


Figure 3.4. Control of spatiotemporal coherence of thalamic spindles by the cerebral cortex in cat. ***Top panel:*** Disruption of the spatiotemporal coherence of thalamic oscillation after removal of the neocortex. Spatiotemporal maps of electrical activity across the thalamus were constructed by plotting time (time runs from top to bottom in each column; arrow indicates 1 s), space (from left to right, the width of each column represents 8 mm in the anteroposterior axis of the thalamus), and local field potential (LFP) voltage [from blue to yellow, color represents the amplitude of the negative deflection of thalamic LFPs; the color scale ranged in 10 steps from the baseline (blue) to −100 μV (yellow)]. Time was divided into frames, each representing a snapshot of 4 ms of thalamic activity. A total of 40 s is represented (9880 frames). Each frame consisted of eight color spots, each corresponding to the LFP of one electrode from anterior to posterior (left to right in each column). ***Middle column (Experiments):*** Decay of correlation with distance. Cross-correlations were computed for all possible pairs of thalamic sites, and the value at time zero from each correlation was represented as a function of the intersite distance for six different consecutive epochs of 20 s. Spatial correlation was calculated for thalamic recordings in the intact brain (left) and after removal of cortex (right). ***Bottom panel (Model):*** Decay of correlation with distance (in units of sites). Similar computation of cross-correlations as in the above panel from experiments, in the presence of cortex (left) and after decortication (right). (Modified from Contreras, D., Destexhe, A., Sejnowski, T.J., and Steriade, M. 1996. Control of spatiotemporal coherence of a thalamic oscillation by corticothalamic feedback. Science 274:771–774; and unpublished data by Destexhe, A. Contreras, D., Sejnowski, T.J., and Steriade, M.)

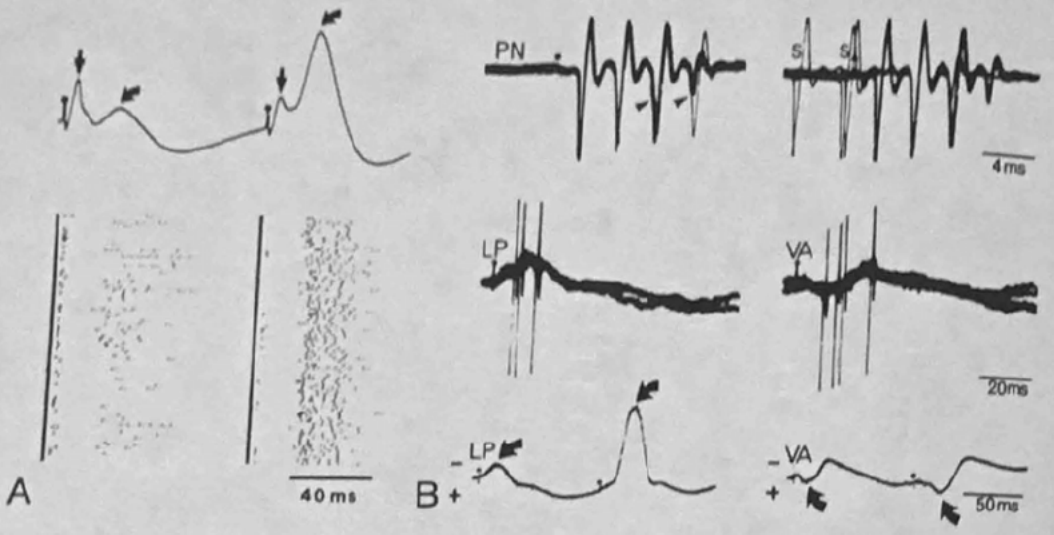


Figure 3.5. Augmenting and recruiting thalamocortical responses. **A.** Simultaneous recording of field potentials (50 averaged sweeps) and unit discharges (dotgrams) at depth of 1 mm in cat anterior suprasylvian gyrus (area 5), evoked by two 0.1-second-delayed stimuli to the lateroposterior thalami nucleus; stimuli indicated by dots (field potential trace) and vertical bars (dotgrams). Primary excitation indicated by straight arrows; secondary excitation indicated by oblique arrows. The secondary depth-negative wave, associated with burst firing in the simultaneously recorded unit, is selectively augmented at second stimulus, whereas the primary excitation is simultaneously depressed. **B.** Augmenting and recruiting response patterns induced in the depth of cortical area 5 in the cat by stimulating lateroposterior (*LP*) thalamic nucleus projecting to middle cortical layers and ventroanterior (*VA*) thalamic nucleus projecting to the superficial layers. Chronically implanted, behaving preparations. Top traces, thalamic-evoked field potentials and unit discharges are from a corticopontine neuron, as antidromically identif stimulation of the pontine nuclei (*PN*); four-shock train at 370 Hz (on artifact of the first shock is indicated by dot); break between initial se and somadendritic spikes (third and fourth responses) or initial segment in isolation (fourth response) indicated by arrowheads; right superimpo shows collision of antidromic responses to the first shock by prece spontaneous (S) discharges. Middle traces, orthodromic discharges ev in the same neuron by stimulating LP or VA thalamic nuclei. Bottom tr field potentials (50 averaged sweeps) evoked by stimulating LP or VA t mic nuclei with two 100-msec-delayed shocks (unit discharges were cut Note augmenting (depth-negative) and recruiting (depth-positive) pat of field responses to stimulation of LP and VA, respectively. (Modi from Steriade, M. 1978. Cortical long-axoned cells and putative interneu during the sleep-waking cycle. Behav. Brain Sci. 1:465–514.)

tro-Alamancos and Connors, 1996a,b). The thalamus can itself generate augmenting responses, as recently demonstrated in intracellular recordings from decorticated animals (Steriade and Timofeev, 1997). In that study, we have described two types of incremental responses to thalamic stimuli: (a) incrementing high-threshold responses occurring on a progressive depolarization of TC cells, associated with the decrease of IPSPs produced by preceding stimuli in the pulse-train; and (b) progressively growing low-threshold responses, resulting from the enhancement of chloride-dependent IPSPs, giving rise to postinhibitory rebound spike-bursts (Fig. 3.6). These two types of intrathalamic augmenting responses of TC cells are the result of changing patterns of repetitive responses in GABAergic RE neurons: high-threshold augmenting responses in TC cells are ascribed to decremental responses in RE cells and, consequently, to disinhibition in their target TC neurons, whereas low-threshold augmenting responses in TC cells are ascribed to incremental responses in RE cells (Steriade and Timofeev, 1996).

Relations between Spindle Mechanisms and Self-sustained Paroxysmal Events of the Spike-and-Wave Type

Several lines of evidence support the idea that the development of spike-and-wave (SW) complexes of the petit mal epileptic type is related to the occurrence of spindles and related oscillations during the state of light sleep, and depen on resonant activities in the reciprocal thalamocorticot lamic loop. (a) SW activity increases during the spindle sta of EEG-synchronized sleep and is attenuated or blocked up arousal (Kellaway, 1985). (b) In the feline generalized pe cillin epilepsy model, spindles develop into bilaterally sy chronous SW complexes and concomitant behavioral un sponsiveness, as in human petit mal attacks (Gloor a Fariello, 1988). (c) Self-sustained SW cortical complexes 2–3 Hz, lasting for 10–15 seconds, may follow thalamocor cal incremental responses (Steriade and Yossif, 1974) or pr longed thalamic stimulation during behavioral drowsiness chronically implanted primates (Steriade, 1974) (Fig. 3. (d) Stimulation of corticothalamic projections within the f quencies range of spindles may lead to self-sustained S complexes in thalamic neurons (Steriade et al., 1976) (Fi 3.8), similarly to the elicitation of cortical SW epileptic co plexes following incremental thalamocortical responses. T idea that the cerebral cortex may lead the thalamus in t generation of SW epilepsy is also supported by the exper ments of Gloor's group (Avoli et al., 1983). Since either t cortex or the thalamus may start the paroxysmal oscillato behavior in the penicillin model of SW epilepsy, it was diff cult to claim that one structure is exclusively playing the ro

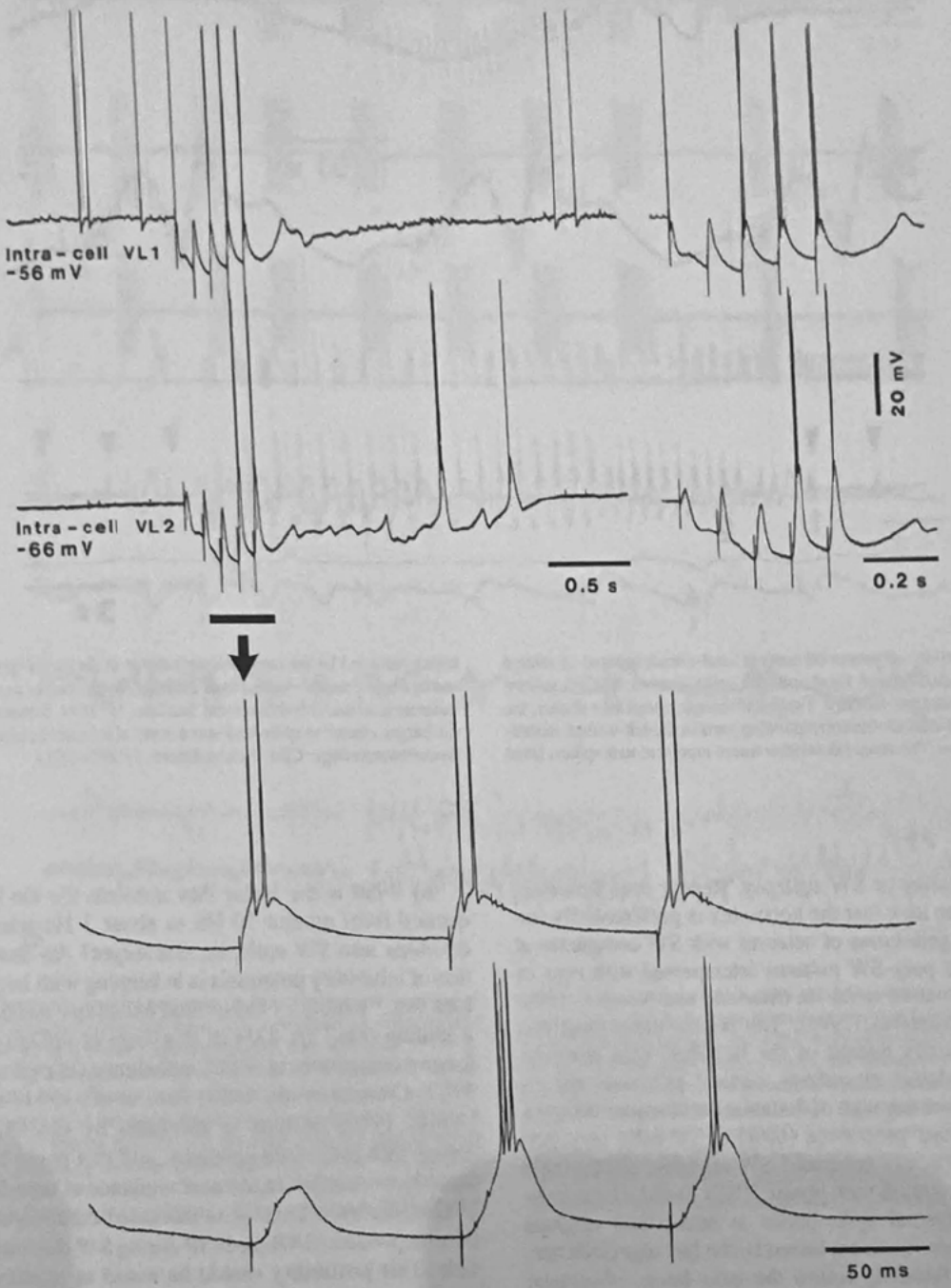


Figure 3.6. Different features (high-threshold and low-threshold) of intrathalamic augmenting responses in the decorticated cat, at depolarized and hyperpolarized levels. Simultaneous dual intracellular recordings of thalamocortical (TC) cells from ventral lateral (*VL*) nucleus. Cell 1 was recorded at a more depolarized membrane potential (−56 mV) than cell 2 (−66 mV). Accordingly, before and after augmenting responses, cell 1 discharged single spikes, whereas cell 2 displayed after augmenting responses a spindle sequence eventually leading to rebound spike-bursts. At right, expanded responses to the 5 stimuli in the train at 10 Hz. The last 3 responses in the train (horizontal bar) are further expanded below (arrow). Note short-latency augmenting responses consisting of spike-doublets in cell 1, whose patterns are quite different from the longer-latency, low-threshold spikes (LTSs) crowned by spike-bursts in cell 2. Small deflections in each of two cells are due to capacitive coupling from action potentials of the other neuron. (From Steriade, M. and Timofeev, I. 1997. Short-term plasticity during intrathalamic augmenting responses in decorticated cats. J. Neurosci. 17:3778-3795.)

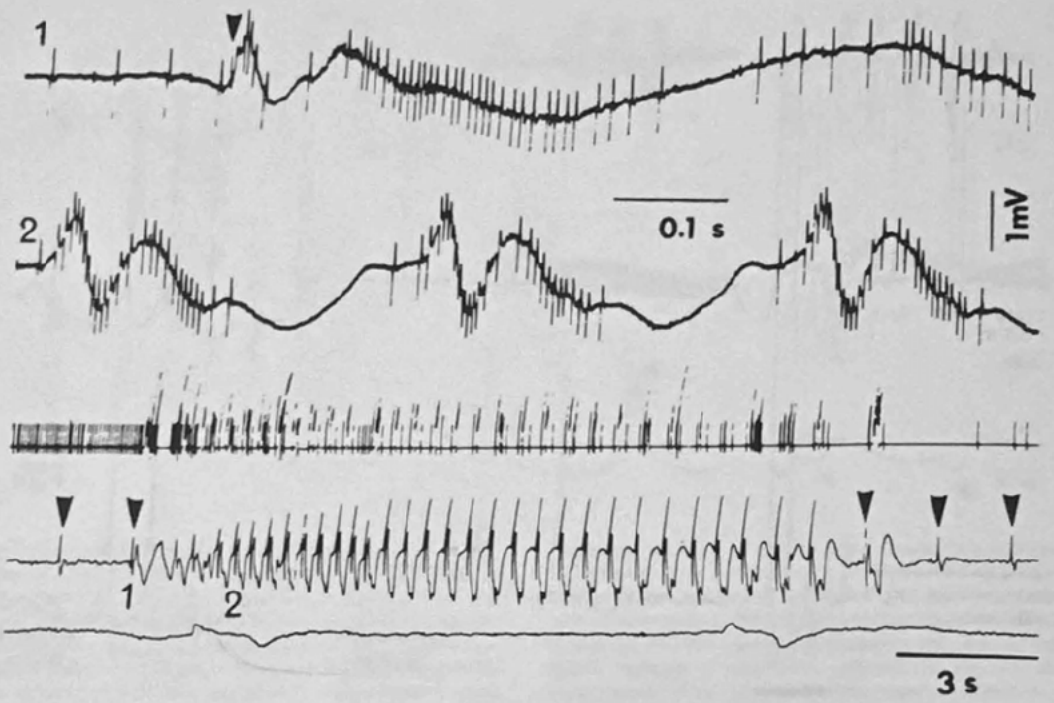


Figure 3.7. Activity of presumed cortical local-circuit neurons is related to the thalamically triggered focal epileptic spike-and-wave (SW) seizure in the behaving macaque monkey. Two oscilloscopic traces area shown; the numbers (1 and 2) indicate the corresponding parts in the ink-written recording depicted below. The three ink-written traces represent unit spikes, focal waves recorded by the same microelectrode in the motor (precentral) corte and eye movements. Arrowheads indicate single shocks to the ventrolater thalamic nucleus. (Modified from Steriade, M. 1974. Interneuronal epilep discharges related to spike-and-wave cortical seizures in behaving monkey Electroencephalogr. Clin. Neurophysiol. 37:247–263.)

of the prime mover in SW epilepsy. Recent data, however, strengthened the idea that the neocortex is preferentially implicated in the generation of seizures with SW complexes at 2–4 Hz or with poly-SW patterns interspersed with runs of fast EEG spikes at 10 to 20 Hz (Steriade and Amzica, 1994; Steriade and Contreras, 1995). The results supporting this hypothesis basically consist of the fact that, with multisite cortical and thalamic recordings, cortical processes are recruited well before any sign of thalamic entrainment. Surprisingly, an important proportion (60%) of TC cells remained silent during cortically generated SW seizures, displaying a tonic hyperpolarization with phasic IPSPs in close time-relation with paroxysmal spike-bursts in neocortical neurons (Fig. 3.9). This result was explained by the fact that GABAergic RE neurons faithfully follow the spike-bursts of cortical neurons and impose IPSPs onto TC cells, thus reinforcing through their exceedingly long spike-bursts the hyperpolarization of TC cells before the latter are able to burst. This was also demonstrated in computer network models of SW seizures (Lytton et al., 1997). Indeed, RE cells discharge spike-bursts of about 40–50 ms during sleep (Domich et al., 1986; Steriade et al., 1986), but increase the duration of their spike-bursts to 200 ms or more during SW seizures (Steriade and Contreras, 1995).

Several questions may be raised as to the relations between the normal (spindles) and the paroxysmal (SW) phenomena.

(a) What is the factor that accounts for the lowered fr quency from around 10 Hz to about 3 Hz when spindli develops into SW epileptic discharges? An increased dur tion of inhibitory potentials is in keeping with Jasper's (196 idea that "inhibitory rather than excitatory mechanisms pl a leading role" (p. 435) in this form of epilepsy. Since t longest component of IPSPs in thalamic (Hirsch and Burno 1987; Crunelli et al., 1988; Paré et al., 1991) and cortic (Avoli, 1986) neurons is mediated by $GABA_B$ recepto linked with potassium channels, and this phase is most su ceptible to changes in state of vigilance (Curró Dossi et a 1992b), the hypothesis of an increased duration of the pota sium-dependent $GABA_B$-IPSP during SW discharges is pla sible. This possibility should be tested as selective GAB blockers are now available, even for systemic administratio The involvement of GABAergic transmission within the th amus in the control of SW seizures was demonstrated in t rat model of genetic absence epilepsy (Liu et al., 1991). Ho ever, the increase in $GABA_B$ IPSPs following blockade $GABA_A$ receptors led to the slowing of the spindle rhyth (7–14 Hz) toward a slower oscillation (2–4 Hz) (von Krosi et al., 1993; D. Contreras and M. Steriade, unpublished), b not to SW seizures, as defined by paroxysmal episodes, sharp contrast with the background activity, and with sudd arrest.

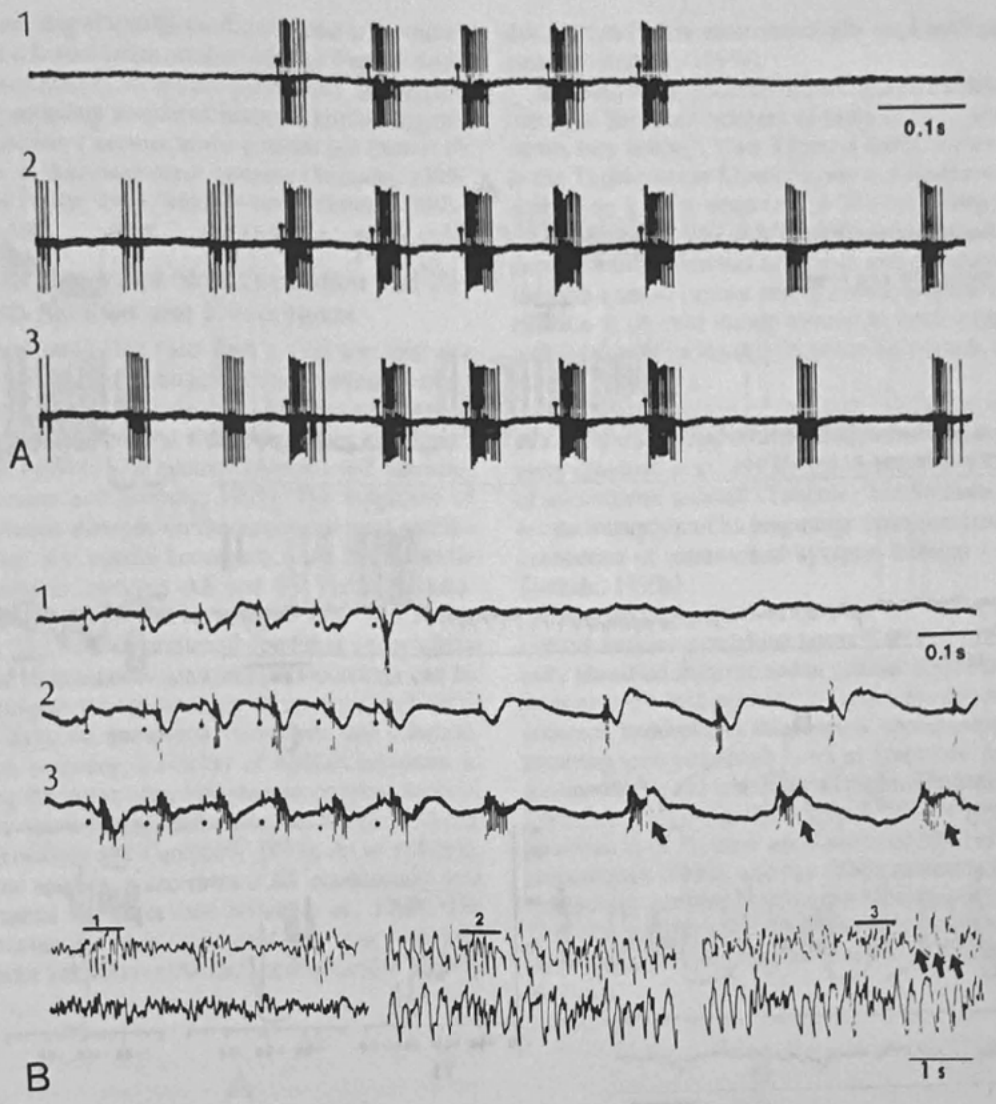


Figure 3.8. Cortically evoked responses in bursting thalamic neurons of cat lead to self-sustained spike-and-wave (SW) complexes. **A.** Ventrolateral cell was driven by motor cortex stimulation with five shocks at 10 Hz (in 1), delivered every 2 seconds. Note the appearance of "spontaneous" bursts that resemble the evoked bursts at a late stage of stimulation (2 and 3). **B.** Effects of stimulation of the suprasylvian area 7 with trains of six shocks at 10 Hz (as in 1) upon a bursting cell recorded from the lateroposterior (LP) nucleus. Beginning with the 12th shock-train, the cell was regularly driven and displayed self-sustained rhythmic bursts at 5 Hz between cortical shock-trains (in 2). Self-sustained SW complexes appeared in 3 (28th shock-train). Bottom, the two ink-written traces represent focal waves in the LP nucleus, recorded by the same microelectrode used for unit recordings (upper trace, negativity upward), and EEG rhythms recorded from the surface of the suprasylvian gyrus (bottom trace). The numbers (1–3) on the EEG recordings correspond to the periods of stimulation depicted with the same numbers in the oscilloscope recordings. (Modified from Steriade, M. 1991. Alertness, quiet sleep, dreaming. In *Cerebral Cortex*, Vol. 9, Eds. A. Peters and E.G. Jones, pp. 279–357. New York: Plenum; Steriade, M., Oakson, G., and Diallo, A. 1976. Cortically elicited spike-wave afterdischarges in thalamic neurons. Electroencephalogr. Clin. Neurophysiol. 41:641–644.)

(b) Knowing that the LTS of thalamocortical neurons is crucial for the patterning of spindles (see above), could blockers of the calcium transient current (I_t) underlying the LTS be effective in the blockage of both spindles and SW epilepsy? It was found that ethosuximide, a drug that is effective in experimental epilepsy and in treating clinical petit-mal seizures, reduces the low threshold calcium current (LTCC) of thalamic neurons in a dose-dependent, reversible manner (Coulter et al., 1989, 1990). It was then expected that ethosuximide blocks not only SW discharges, but also spindling. Pellegrini et al. (1989) demonstrated that, in cat, the effect of ethosuximide on RE thalamic cells is twofold: a marked reduction in the occurrence of RE-cells' spike barrages; and a tendency of RE-cells' bursts to shift toward tonic firing. It is known that spike-bursts in RE neurons are correlated with spindling, whereas the tonic firing of the same cells characterizes brain-activated, EEG-desynchronized behavioral states (Steriade et al., 1986). Thus, ethosuximide would reduce inhibitory processes within the RE network (responsible for spindle genesis) as well as the LTCC of thalamocortical cells

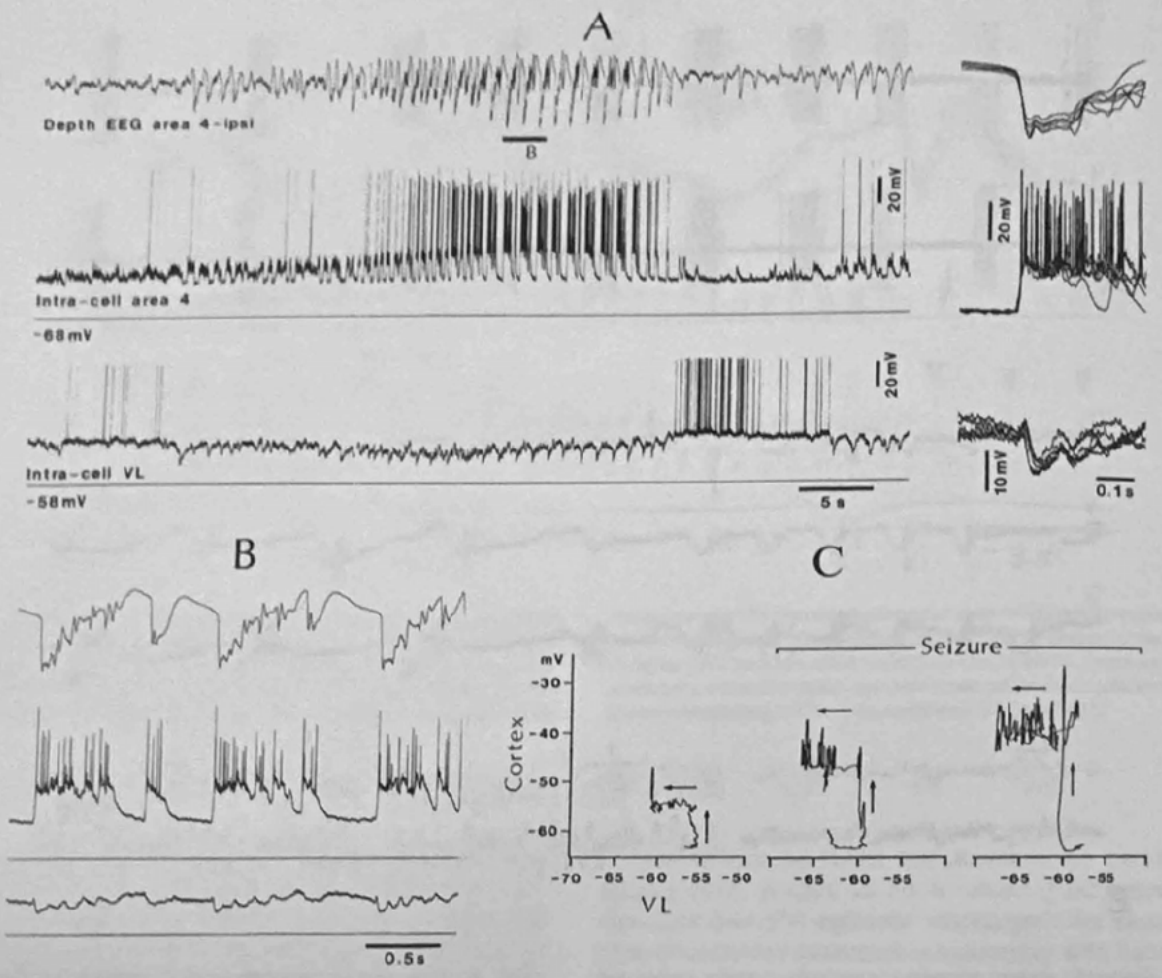


Figure 3.9. Dual intracellular recordings demonstrating hyperpolarization of thalamocortical (TC) cell in the ventrolateral (*VL*) nucleus during seizure depolarization and spike-bursts in area 4 cortical neuron. Cat under ketamine–xylazine anesthesia. **A.** Three traces depict simultaneous recording of depth-EEG from area 4, as well as intracellular activities of area 4 cortical neuron and TC cell from the ipsilateral VL nucleus (below each intracellular trace, current monitor). The seizure was initiated by a series of EEG waves at about 0.9 Hz in the depth of area 4, continued with discharges at about 2 Hz, and ended with high-amplitude, periodic (0.9 Hz) EEG sequences consisting of wavelets at 14 Hz. All these periods were faithfully reflected in the intracellular activity of cortical neuron, whereas VL thalamic neuron displayed a tonic hyperpolarization throughout the seizure, with phasic sequences of IPSPs related to the large cortical paroxysmal depolarizations and spike-bursts occurring at the end of the seizure. Note disinhibition of VL cell after cessation of cortical seizure. At right, superimposition of six successive, expanded traces from the part indicated by horizontal bar (**B**) and continuing with subsequent three polyspike-wave complexes. Note spiky depth-negative EEG deflection associated with depolarization of cortical ce and rhythmic IPSPs of VL thalamic neuron. Part marked by *B* is furth depicted in the bottom left panel. **C.** Phase relations between simultaneousl intracellularly recorded area 4 cortical neuron and VL thalamic neuron a preserved during sleep and development of seizure activity. The three pa represent (from left to right): one period before seizure, during EEG sle patterns; and two periods during early and late parts of the seizure. Pha plots of averaged membrane voltage of area 4 cell (ordinate) against that VL cell (abscissa). The development of seizure did not change the phas relation between cells, but accentuated the amplitude of the elements cons tuting the normal (sleep) oscillatory behavior preceding the seizure. In c sence, cortical depolarization (upward arrows) preceded VL-cell's hyperp larization (left-directed arrows) in the three periods, although the amplitu of the membrane excursions was considerably enhanced during the seizu (Modified from Steriade, M. and Contreras, D. Relations between cortic and thalamic cellular events during transition from sleep patterns to paroxy mal activity. 1995. J. Neurosci. 15:623–642; and unpublished data).

(involved in patterning of spindle oscillations and in the transfer of rebound excitation to the cerebral cortex). Further studies, with measurements of all spindle parameters, are needed to explore the promising avenue of research implicating the RE thalamic inhibitory nucleus in the genesis and control of SW discharges in thalamocortical systems (Steriade, 1990; Huguenard and Prince, 1994; Steriade and Contreras, 1995; Lytton et al., 1997).

The Cortically Generated Slow Oscillation and Its Relations with Spindles and K-complexes

A novel slow oscillation (less than 1 Hz) was first described in intracellular recordings from neocortical neurons in anesthetized animals (Steriade et al., 1993e) and was subsequently found during natural slow-wave sleep of animals (Steriade et al., 1996a) and humans (Amzica and Steriade, 1997a; Achermann and Borbély, 1997). The frequency of the slow oscillation depends on the anesthetic used and the behavioral state: it is mainly between 0.3 and 0.6 Hz under urethane anesthesia; between 0.6 and 0.9 Hz under ketamine–xylazine anesthesia; and between 0.7 and ~1 Hz during natural sleep. The best experimental condition under which single or dual simultaneous intracellular recordings can be made to investigate the mechanisms of the slow oscillation is ketamine–xylazine anesthesia (Contreras and Steriade, 1995). Indeed, ketamine, a blocker of NMDA receptors, is placed among the most effective pharmacological tools in inducing slow-wave sleep patterns over the background of a wake state (Feinberg and Campbell, 1993). As to xylazine, an α_2-receptor agonist, it increases a K^+ conductance in a variety of central structures (see Nicoll et al., 1990). The similarity between the slow oscillation occurring in natural sleep and under ketamine–xylazine anesthesia was recently demonstrated in the same chronically implanted animal (Amzica and Steriade, 1997b).

It should be emphasized that the slow oscillation does not belong to the same category of brain rhythms as sleep delta waves (see below, "Two Types of Delta Waves Generated in the Thalamus and Cortex"), nor is it similar to the cyclic alternating pattern recurring at 20-sec or longer intervals (Terzano et al., 1988). The latter is associated with enhancement of muscle tone and heart rate, and was described under the term *arousal-related phasic events*, whereas the slow oscillation is blocked during arousal in acute experiments as well as during awakening in behaving animals (Steriade et al., 1993a, 1996a).

The cortical nature of the slow oscillation was demonstrated by (a) its survival in the cerebral cortex after thalamectomy (Steriade et al., 1993f); (b) its absence in the thalamus of decorticated animals (Timofeev and Steriade, 1996); and (c) the disruption of its long-range synchronization after disconnection of intracortical synaptic linkages (Amzica and Steriade, 1995b).

Intracellular analyses of the slow oscillation showed that cortical neurons throughout layers II to VI (with physiologically identified thalamic and/or callosal inputs, and with projections to the thalamus and/or homotopic points of the contralateral hemisphere) displayed a spontaneous oscillation recurring with periods of 1–1.5 to 5 seconds, depending on the anesthetic, and consisting of prolonged depolarizing and hyperpolarizing components (Fig. 3.10). The long-lasting depolarization of the slow oscillation consisted of EPSPs, fast prepotentials (FPPs), and fast IPSPs reflecting the action of synaptically coupled GABAergic local-circuit cortical cells. Data also indicated that the depolarizing component is made up of both NMDA-mediated synaptic excitatory events and

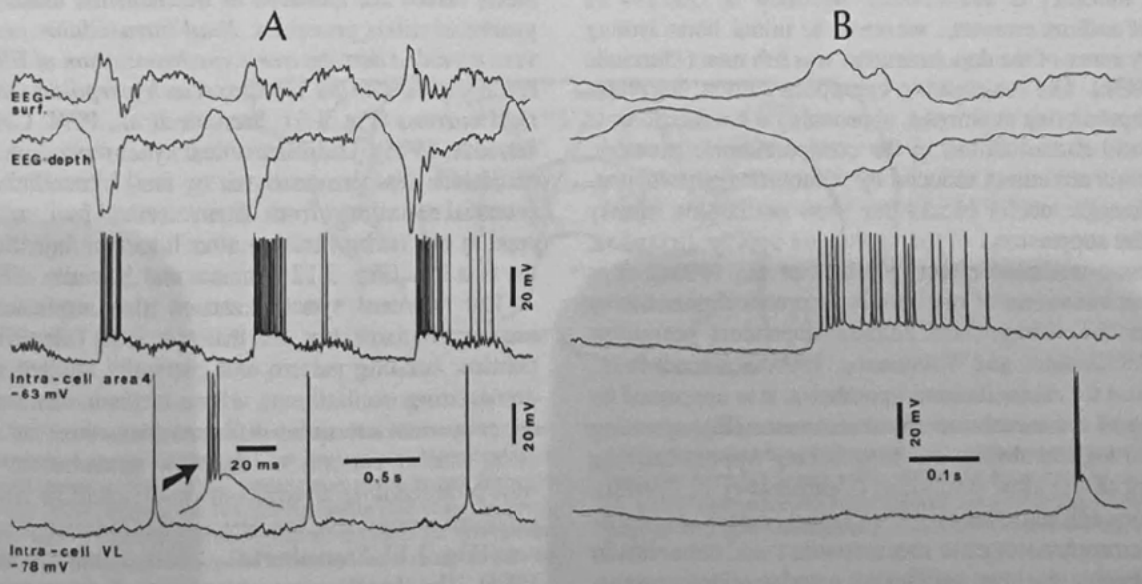


Figure 3.10. The slow oscillation (about 0.9 Hz) in dual simultaneous intracellular recordings from regular-spiking cell in cortical area 4 and thalamocortical cell in the ventrolateral nucleus. Cat under ketamine–xylazine anesthesia. Arrow in A points to a low-threshold spike-burst. An expanded cycle is shown in B. Note: (a) Depth-positive (upward) EEG waves are associated with hyperpolarization of cortical and thalamic cells, whereas the sharp depth-negatives are associated with depolarization and action potentials in cortical cell, while the thalamic neuron displays a rebound spike-burst with a delay of 150–200 ms; (b) brief sequence of EEG spindles after the depth-negative (surface-positive) sharp deflection; and (c) fast depolarizing waves (40–50 Hz) in cortical neuron during the sustained depolarization. Unpublished data by M. Steriade and D. Contreras.

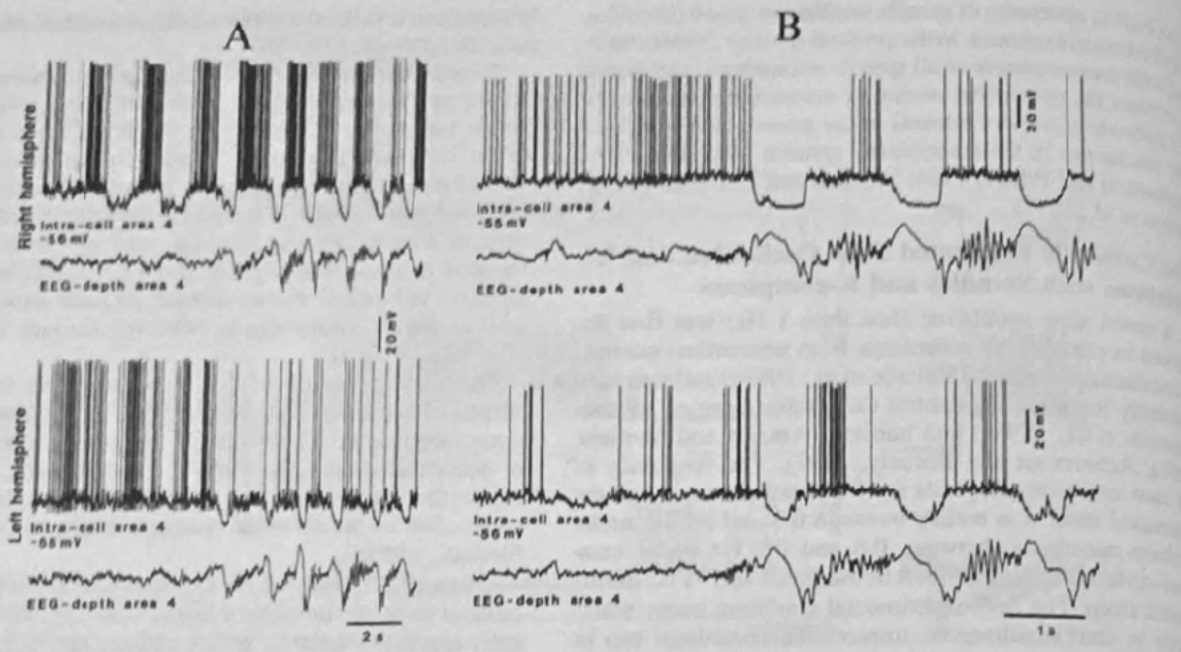


Figure 3.11. The synchronization of EEG is concomitant with simultaneous hyperpolarizations in neocortical neurons. Dual intracellular recordings from right and left areas 4. Two neuronal couples (**A** and **B**) are shown. Note in **A** that, while right area 4 neuron started to display low-amplitude, rhythmic hyperpolarizations, left area 4 neuron still displayed tonic firing, and the EEG showed an activated pattern; only when both cells simultaneously displayed large hyperpolarizations, was the EEG fully synchronized with the patterns of slow oscillation and brief spindle sequences. In **B**, simultaneous hyperpolarizations in the two neurons occurred suddenly. (Modified from Steriade, M., Contreras, D., and Amzica, F. 1994. Synchronized sleep oscillations and their paroxysmal developments. 1994. Trends Neurosci. 17: 199–208. Contreras, D. and Steriade, M. 1995. Cellular basis of EEG slow rhythms: a study of dynamic corticothalamic relationships. 1995. J. Neurosci. 15:604–622.)

a voltage-dependent persistent sodium current $g_{Na(p)}$. This was suggested by the suppression of the prolonged part of the depolarizing envelope by either administration of ketamine (NMDA blocker) or intracellular injection of QX-314 (a blocker of sodium currents), whereas the initial, short-lasting excitatory event of the depolarization was left intact (Steriade et al., 1993e). The long-lasting hyperpolarization, interrupting the depolarizing envelopes, is probably a combination of a $g_{K(Ca)}$ and disfacilitation in the corticothalamic network. Indeed, brain activation induced by stimulation of mesopontine cholinergic nuclei blocks the slow oscillation mainly through the suppression of the prolonged hyperpolarization, a muscarinic-mediated effect (Steriade et al., 1993a); it is known that activation of muscarinic receptors diminishes or suppresses the voltage- and calcium-dependent potassium current (McCormick and Williamson, 1989; Schwindt et al., 1989). As to the disfacilitation hypothesis, it is supported by measuring of the membrane input resistance (R_{in}) showing that R_{in} is highest during the long-lasting hyperpolarizing component of the slow oscillation (Contreras et al., 1996b).

All major cellular classes in the cerebral cortex, as identified by electrophysiological characteristics and intracellular staining, display the slow oscillation: regular-spiking and intrinsically bursting cells, as well as local-circuit inhibitory basket cells (Steriade et al., 1993a,e; Contreras and Steriade, 1995). All these neuronal types exhibit similar relations with the EEG components of the slow oscillation: during the depth-positive EEG wave cortical neurons are hyperpolarized, whereas during the sharp depth-negative EEG deflection cortical neurons are depolarized (Fig. 3.10). The spectacular similarity between all types of cortical neurons and EEG waveforms raised the question of mechanisms underlying these synchronization processes. *Dual intracellular recordings in vivo revealed that the overt synchronization of EEG patterns is associated with the simultaneous hyperpolarizations in cortical neurons* (Fig. 3.11; Steriade et al., 1994; Contreras and Steriade, 1995). The intracortical synchronization of the slow oscillation was demonstrated by dual intracellular and field potential recordings from distant cortical foci, and by the disruption of synchronization after lidocaine injection between the two foci (Fig. 3.12; Amzica and Steriade, 1995b).

The neuronal synchronization also implicates thalamic neurons. Remarkably, RE thalamic cells (identified by their peculiar bursting pattern and cortically elicited spindle-like depolarizing oscillations), whose intrinsic electrophysiological properties are quite different from those of neocortical cells, exhibit patterns of the slow spontaneous oscillation with prolonged depolarizations interrupted by prolonged hyperpolarizations, that are very similar to those of cortical neurons (Fig. 3.13; Steriade et al., 1993b; Contreras and Steriade, 1995). The depolarizing component of the cortically generated slow oscillation is transmitted to RE thalamic neurons at which level it triggers rhythmic spike-bursts and, consequently, is reflected in TC cells as rhythmic IPSPs leading to rebound spike-bursts (Fig. 3.14). *This is the mechanism underlying the brief sequence of spindles that follows every*

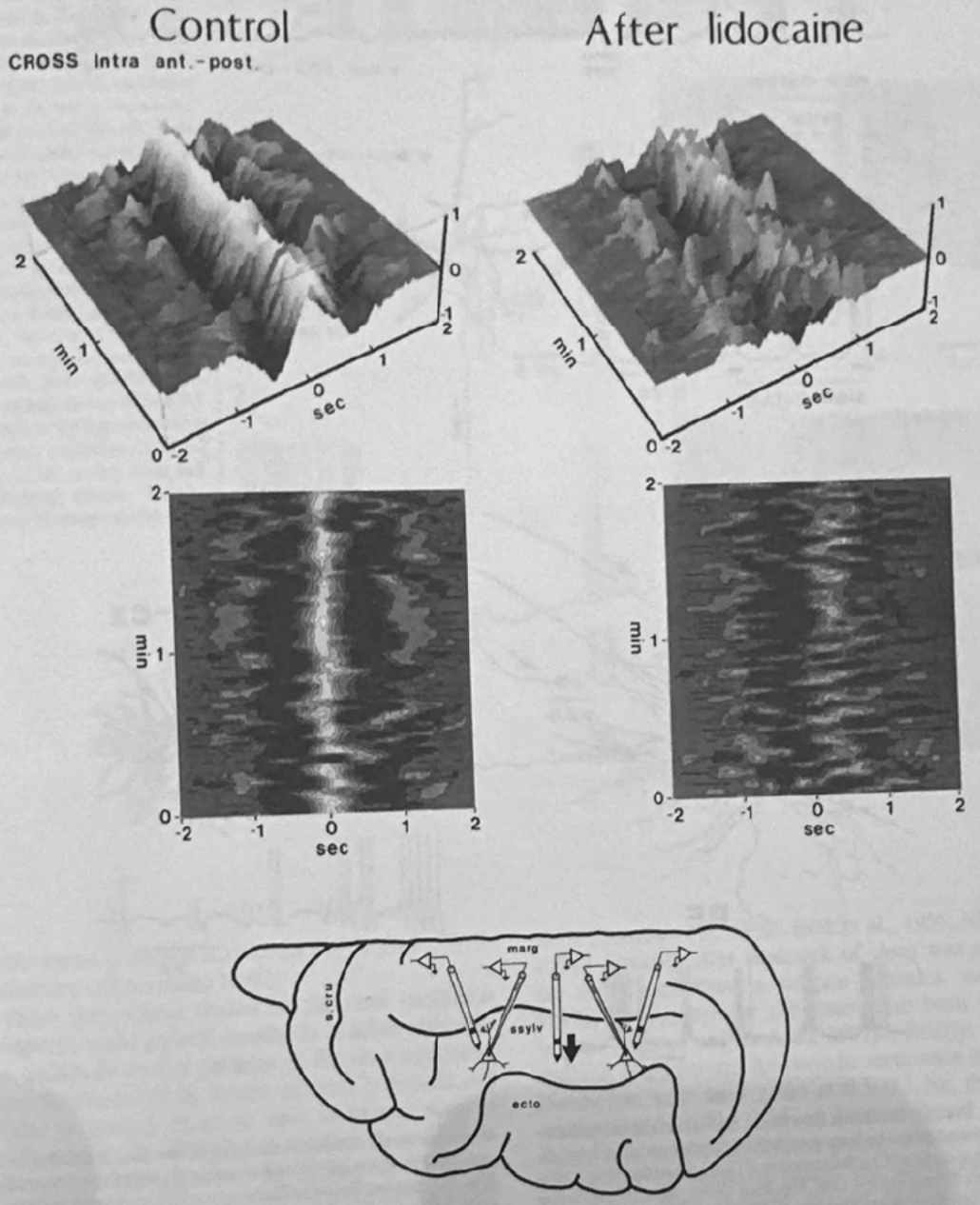


Figure 3.12. Disruption of synchronization of slow oscillation by intracortical disconnection of synaptic linkages. Dual intracellular recordings from the anterior and posterior parts of the suprasylvian gyrus in cat; lidocaine injection (40 μl, 20%) between the two micropipettes (see brain figurine). The synchrony and its disruption after lidocaine injections are represented by sequential field analyses. The control synchrony was characterized by well-aligned, high central peaks. After lidocaine injection, the previous pattern was replaced by a blurred sequence of lower peaks and lower valleys deviating from the central plane. (Modified from Amzica, F. and Steriade, M. 1995b. Disconnection of intracortical synaptic linkages disrupts synchronization of a slow oscillation. J. Neurosci. 15:4658–4677.)

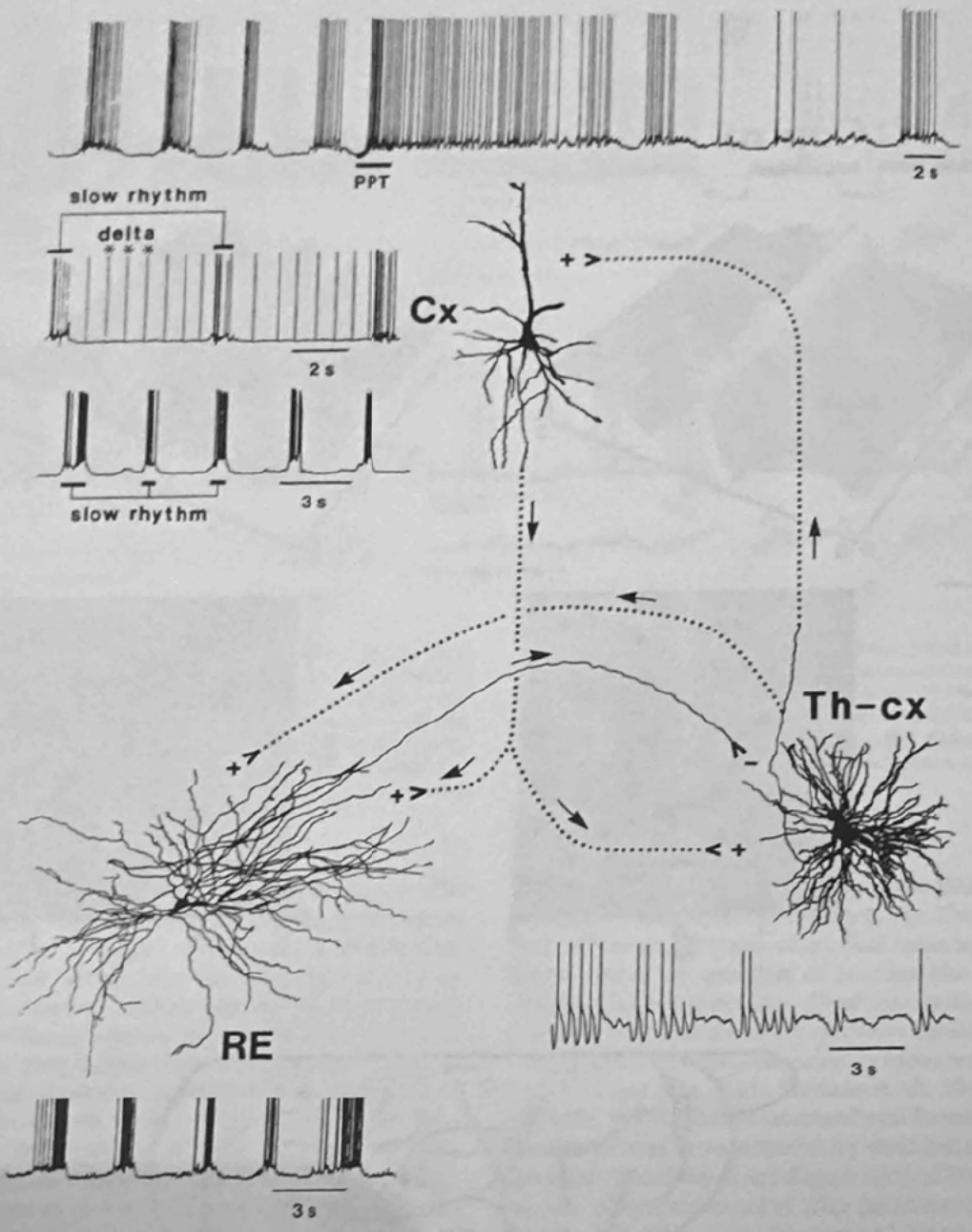


Figure 3.13. The slow oscillation (less than 1 Hz) in cortical and thalamic neurons, and its modulation by brainstem core cholinergic systems. Intracellular recordings of cortical (*Cx*), thalamocortical (*Th-cx*), and thalamic reticular (*RE*) neurons in anesthetized cats. Top trace shows the slow oscillation (0.3 Hz) of Cx cell from association area 5. A brief pulse-train to the brainstem cholinergic pedunculopontine tegmental (*PPT*) nucleus (horizontal bar) transiently suppressed the slow cortical oscillation and replaced it by tonic firing. The effect was associated with an EEG-activated response that had a similar time-course, and was blocked by a muscarinic antagonist, scopolamine (not shown). Below, a summary diagram depicting several aspects of the cortically generated slow oscillation in interconnected cortical and thalamic networks. The Cx neuron was stained intracellularly with Lucifer yellow, and the RE and Th-cx neurons were stained intracellularly with horseradish peroxidase. The direction of the axons is indicated by arrows, and the excitatory or inhibitory signs are indicated by + or −. The two Cx neurons, recorded from association area 5, show a slow rhythm (0.17 Hz in upper trace, 0.3 Hz in lower trace). In top trace, note the appearance, between the depolarizing envelopes of the slow cortical rhythm, of clocklike action potentials (asterisks) recurring at the delta frequency (1.5 Hz) and generated in Th-cx cells. The RE neuron displayed the slow oscillation (0.3 Hz). The delta oscillation in Th-cx neuron (2.5 Hz) tended to dampen periodically within the frequency range of the slow cortical rhythm (0.2–0.3 Hz) because of an increase in membrane conductance resulting from converging excitatory inputs arising in Cx cells and inhibitory inputs arising in GABAergic RE neurons. (Modified from Steriade, M., Amzica, F., and Nuñez, A. 1993. Cholinergic and noradrenergic modulation of the slow (~0.3 Hz) oscillation in neocortical cells. J. Neurophysiol. 70:1385–1400; Steriade, M., Nuñez, A., and Amzica, F. 1993. A novel slow (<1 Hz) oscillation of neocortical neurons *in vivo*: depolarizing and hyperpolarizing components. J. Neurosci. 13:3252–3265; Steriade, M., Nuñez, A., and Amzica, F. 1993. Intracellular analysis of relations between the slow (<1 Hz) neocortical oscillation and other sleep rhythms of the electroencephalogram. J. Neurosci. 13:3266–3283; Steriade, M., Contreras, D., Curró Dossi, R., and Nuñez, A. 1993. The slow (<1 Hz) oscillation in reticular thalamic and thalamocortical neurons: scenario of sleep rhythm generation in interacting thalamic and neocortical networks. J. Neurosci. 13:3284–3299.)

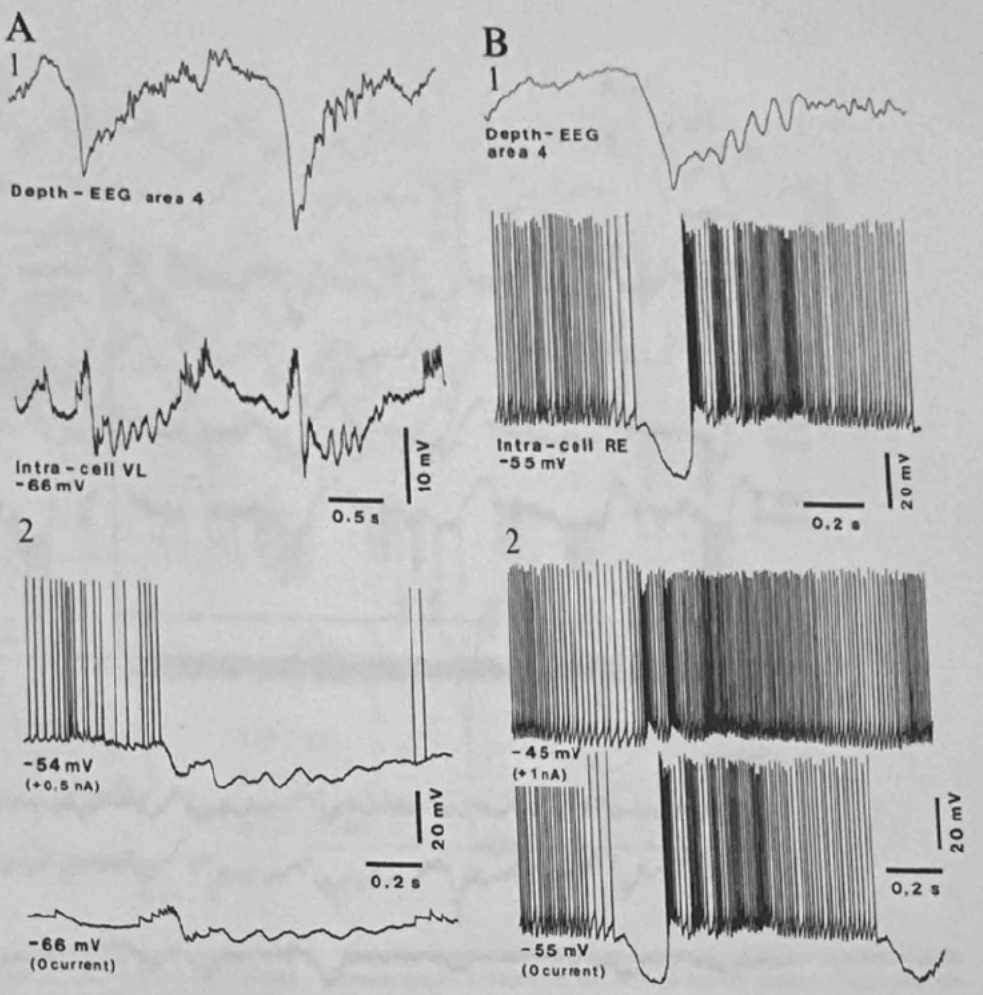


Figure 3.14. Effect of sharp cortico-thalamic volleys during the depolarizing phases of the slow sleep oscillation upon thalamocortical and thalamic reticular cells. Cat under ketamine–xylazine anesthesia. **A.** Depth-EEG from area 4 and intracellular recording from ventrolateral (*VL*) thalamocortical neuron. In 1, two cycles of slow oscillation (0.5–0.6 Hz) at the resting membrane potential (−66 mV) of VL cell. Note brief sequence of spindle waves in EEG and corresponding IPSPs in VL cell following the sharp depth-EEG (depolarizing) component of the slow oscillation. In 2, expanded traces to illustrate enhancement of spindle-related IPSPs with cells depolarization. **B.** Depth-EEG from area 4 and intracellular recording from thalamic reticular (*RE*) neuron. In 1, resting membrane potential (−55 mV). Note spindle-related spike-bursts in association with EEG spindles that follow the hyperpolarizing phase of the slow oscillation. In 2, expanded trace at the resting level and under depolarizing current. Unpublished data by I. Timofeev and M. Steriade.

cycle of the slow oscillation (Contreras and Steriade, 1995, 1996; Timofeev and Steriade, 1996).

The above intracellular studies on the slow oscillation were conducted under general anesthesia to allow stable recordings. Strikingly similar patterns of the slow oscillation have been demonstrated by means of field potentials and extracellular recordings of single units in natural sleep of animals (Steriade et al., 1996a) and, through scalp EEG recordings, in human slow-wave sleep (Amzica and Steriade, 1997a; Achermann and Borbély, 1997). The resemblance between the pattern of slow oscillation during natural sleep and anesthesia concerns the two major components of this rhythm: the prolonged hyperpolarization during the depth-positive EEG wave is associated with silent firing, and the depolarizing component during the depth-negative EEG wave is accompanied by brisk firing that eventually leads to a sequence of spindle waves and to a depolarizing plateau associated with fast oscillations (Fig. 3.15).

The sequence of grapho-elements consisting of an ample surface-positive transient followed by a slower, surface-negative component and, eventually, a few spindle waves is usually termed the K-complex and is a reliable sign for stage 2 of human sleep, but surviving in all stages of quiet sleep (Loomis et al., 1938; Roth et al., 1956; Niedermeyer, 1993). Recently, this landmark of sleep was related with the slow oscillation in humans (Amzica and Steriade, 1997a) and its cellular substrates have been investigated in cats (Amzica and Steriade, 1997b). Briefly: (a) spectral analysis demonstrated the periodic recurrence of human K-complexes, with main peaks at 0.5–0.7 Hz; the other frequency bands in Fig. 3.16 are between 1 and 4 Hz (delta band, with several ill-defined peaks) and between 12 and 15 Hz for the spinding range; the decomposition of the signal into three digitally filtered channels (Fig. 3.16D) indicates that the S-lead reflects the slow oscillation, the lead reflects the shape of the K-complex, and the lead faithfully reflected the spindle activity of the original signal; (b) the laminar profile and intracellular substrates of the K-complex during cat sleep or anesthesia revealed that the surface-recorded, positive K-complexes reverse at a cortical depth of about 0.3 mm, and that the sharp depth-negative (surface-positive) wave of the K-complex is associated with cells' depolarizations, eventually leading to a spindle sequence. These investigations indicate that the K-complex are the expression of the spontaneously occurring, cortically generated slow oscillation.

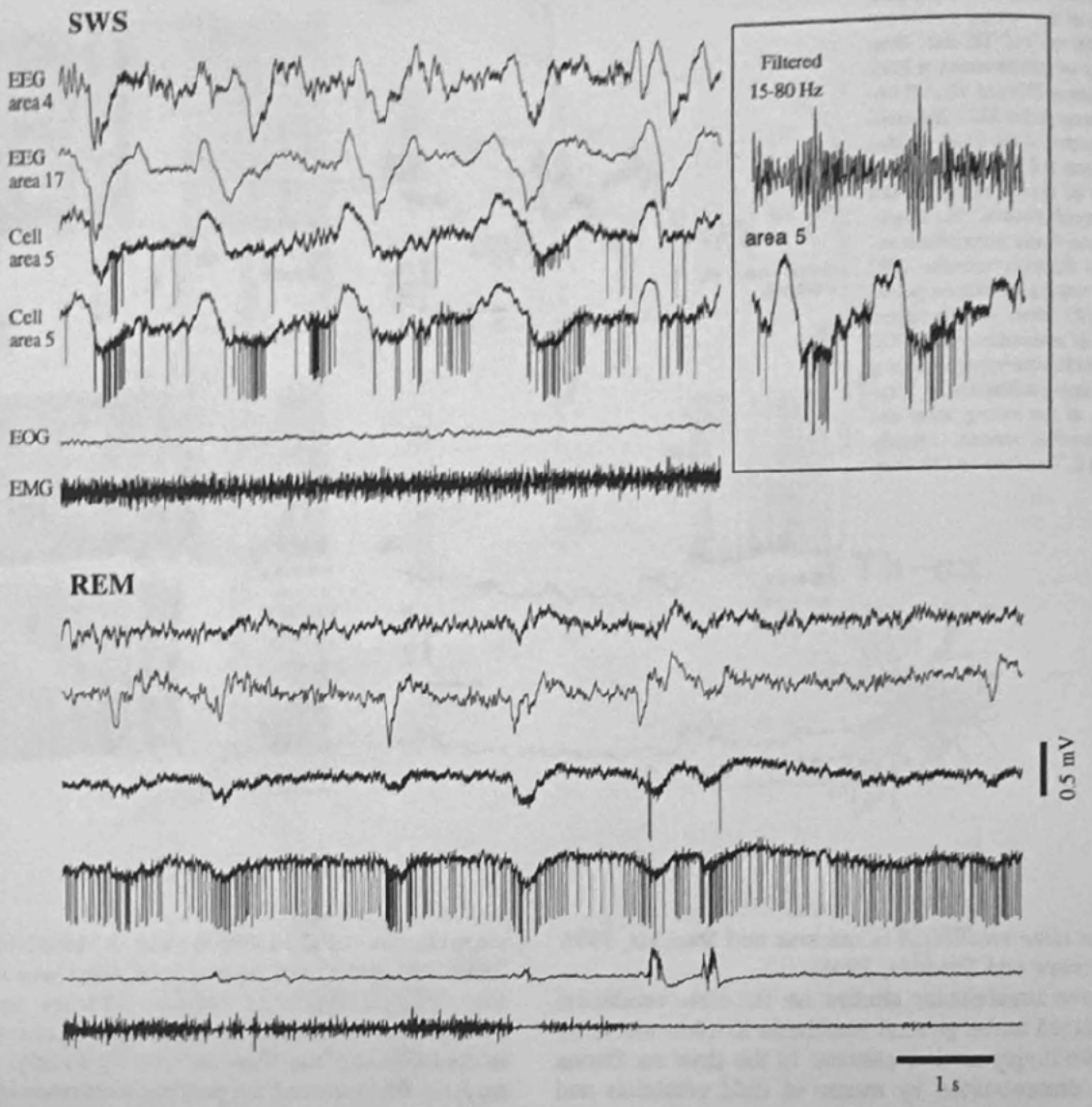


Figure 3.15. Patterns of slow oscillation during natural sleep are similar to those under ketamine–xylazine anesthesia. Selective reduction of fast rhythms during the inhibitory periods of the slow oscillation during natural sleep. Chronically implanted, naturally sleeping cat. *SWS*, slow wave sleep. *REM*, rapid-eye-movement sleep. SWS is separated from REM by a nondepicted period of 34 s. Six traces represent: depth-EEG from motor (precruciate) area 4; depth-EEG from visual area 17; unit discharges and slow focal potentials from association area 5 in the anterior suprasylvian gyrus; and similar recording from an adjacent focus (2 mm apart) in area 5; electrooculogram (EOG); and electromyogram (EMG). Right part in SWS panel shows reduction, up to disappearance, of fast rhythms (filtered 15–80 Hz) during the prolonged depth-positive wave of the slow oscillation that, in intracellular recordings, is associated with hyperpolarization of cortical and thalamic neurons. (Unpublished data by M. Steriade and F. Amzica; inset in SWS is adapted from Steriade, M., Amzica, F., and Contreras, D. 1996. Synchronization of fast (30–40 Hz) spontaneous cortical rhythms during brain activation. J. Neurosci. 16:392–417.)

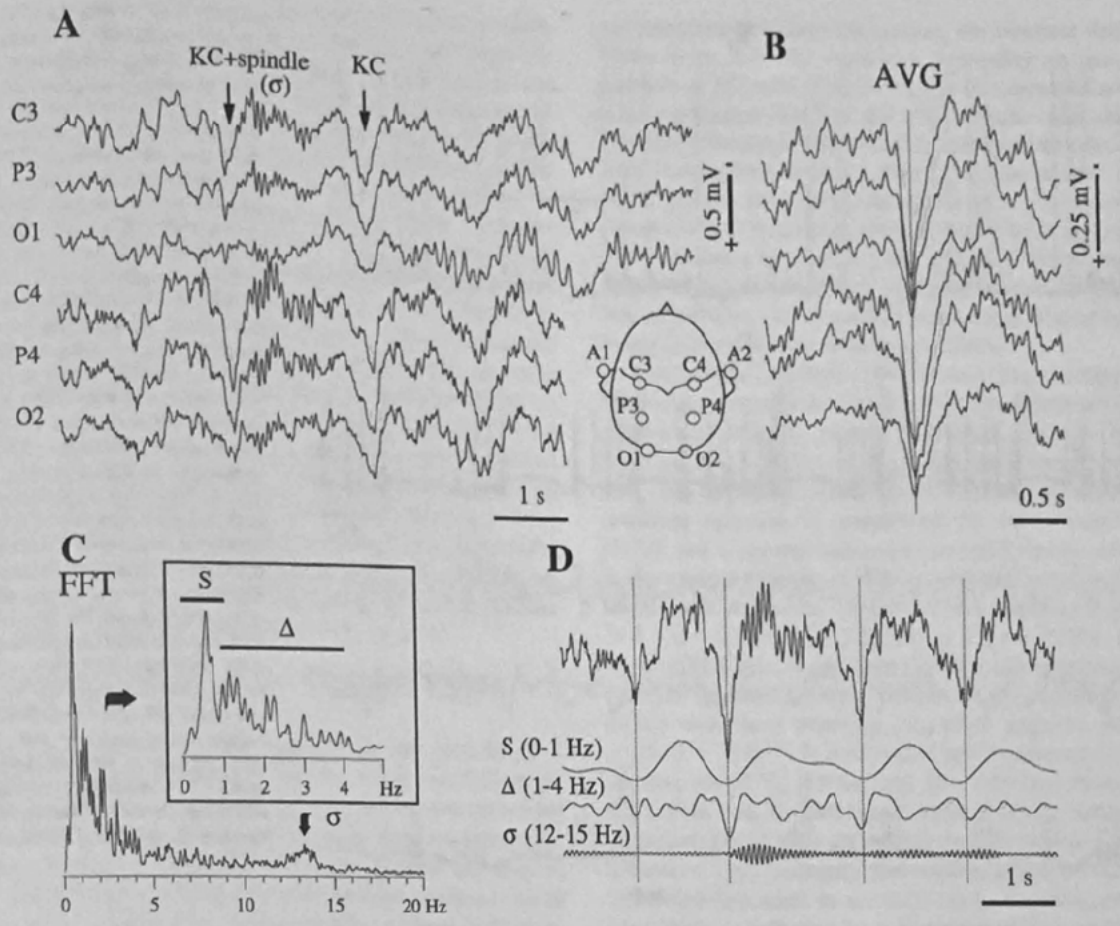


Figure 3.16. K-complex in human sleep. Scalp monopolar recordings with respect to the contralateral ear (see figurine). **A.** Short episode from a stage 3 period of sleep. The two arrows point to a K-complex (*KC*) followed by a spindle sequence (δ) and to a KC in isolation. The two KCs are embedded into a slow oscillation at about 0.6 Hz. Note the synchrony of KCs in all recorded sites and the diminution of their amplitudes in the occipital area. **B.** Average of 50 KCs aligned on the positive peak of the upper channel (vertical dotted line). **C.** Power spectrum of the C3-lead for a period of 80 s of stable stage 3 activity containing the period depicted in **A**. The three frequency bands (S,Δ, and δ; further illustrated in **D**) are represented in the power spectrum. Moreover, the slow (*S*) activity displays a high peak, distinct from the onset of delta (Δ) activity (see inset). **D.** Frequency decomposition of the C3-lead (upper trace) into three frequency bands: slow (S; 0.1 Hz); delta (Δ; 1–4 Hz); and sigma (δ; 12–15 Hz). It is shown that the KC results from a combination of slow and delta waves. (From Amzica, F. and Steriade, M. 1997. The K-complex: its slow (<1 Hz) rhythmicity and relation with delta waves. Neurology. 49:952–959.)

Two Types of Delta Waves Generated in the Thalamus and Cortex

As sleep deepens, the incidence of spindles diminishes and high-amplitude, slower EEG rhythms progressively develop. These waves are conventionally termed *delta*, whence the term *delta sleep* applied to stages 3–4 of sleep in humans or to the later part of EEG-synchronized sleep in cats. A major task was to explore the cellular mechanisms that underlie the recently discovered slow oscillation (less than 1 Hz), described above, distinct from those that built up the conventional delta waves (1 to 4 Hz). Hereafter, the term *delta* will be conventionally used for oscillations within the frequency range of 1–4 Hz, and the term *slow* for waves below 1 Hz.

The difference between these two oscillatory types is emphasized because of their distinct origins and mechanisms (the slow oscillation emerges from network activity in the cerebral cortex, whereas at least one type of delta activity arises from the interplay between two intrinsic currents of thalamic cells) and because delta waves are grouped by the slow oscillation (Fig. 3.17; Steriade et al., 1993f). This underlines the fact that we deal with two different sleep oscillations. With the benefit of hindsight, one can see in previous EEG recordings cyclic groups of delta waves at 3–4 Hz recurring with a slow rhythm of 0.3–0.4 Hz in human sleep (Niedermeyer, 1993) and recent analyses of human sleep have also indicated the differences between the slow and delta oscillations (Achermann and Borbély, 1997).

There are two types of delta activity. One is generated in the cortex, as it survives after thalamectomy, but its neuronal substrates have not yet been systematically investigated with

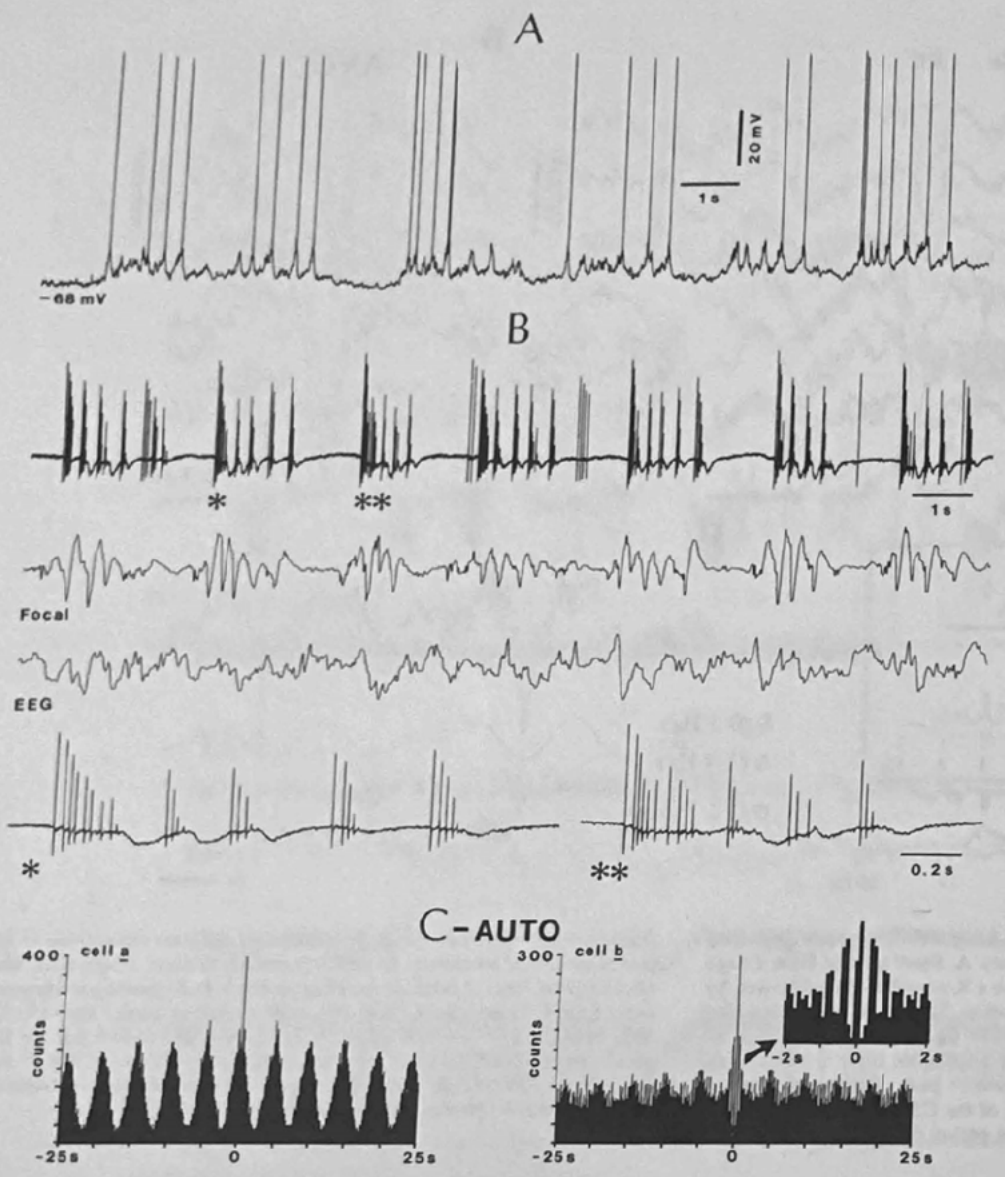


Figure 3.17. Grouping of delta waves by slow oscillation. Cats under urethane anesthesia. **A.** Intracellular recording of regular-spiking, corticothalamic cell in area 5, backfired from rostral intralaminar central lateral (CL) and driven synaptically from lateral posterior (LP) nucleus. Note delta cellular oscillation (3–4 Hz) grouped within sequences recurring with a slow rhythm (0.3–0.4 Hz). **B.** Extracellular recording of bursting cell at a depth of 0.6 mm in area 7, convergently excited by LP and CL nuclei. Below the cellular trace, focal waves recorded through the same micropipette and gross EEG waves from the cortical surface are depicted. The sequence of spike-bursts marked by one and two asterisks are expanded below. Delta oscillations (3–4 Hz) are grouped by the slow oscillation (0.3–0.4 Hz). **C.** Slow and delta rhythms in two cells, recorded simultaneously, extracellularly (*a*, single-spike discharging, and *b*, bursting cell) from motor area 4 (depth 1.3 mm). Autocorrelograms show that both cells displayed the slow oscillation (0.2) and that cell *b* also oscillated in the delta frequency (2.5 Hz) within the slowly recurring sequences (see arrow and inset). (Modified from Steriade, M., Nuñez, A., and Amzica, F. 1993. Intracellular analysis of relations between the slow (<1 Hz) neocortical oscillation and other sleep rhythms of the electroencephalogram. J. Neurosci. 13:3266–3283.)

intracellular recordings. The other originates in the thalamus, even after decortication, and its cellular mechanisms are quite well understood.

Early Studies

Until recently little, if anything, was known about the origin and neuronal substrates of delta EEG waves. Their cortical origin was suggested on the basis that delta waves, at 1–2 Hz, survive on the EEG of athalamic cats (Villablanca, 1974). However, while "most of the thalamus was removed," in some animals the posterior parts were spared and "the rostral thalamus was only partially removed" (Villablanca, 1974, pp. 55–57). The thalamectomy by aspiration through the midline using a transcallosal approach in order to minimize direct cortical damage is heroic surgery and may leave intact important thalamic territories; even if only parts of the rostral intralaminar wing are spared, the widespread cortical projections of these nuclei may transfer to the cortex thalamically generated delta oscillations. In fact, thalamic recordings have shown the presence of focal delta waves even after cortical disconnection (see below).

As to the electrophysiological mechanisms, no intracellular data related to delta waves were available until the early 1990s. Stratigraphic studies (Calvet et al., 1964) and current source density analyses (Petsche et al., 1984) have indicated that delta waves result from vertically arranged dipoles between layers II–III and layer V. Extracellular recordings of cortical activity during pathological delta waves (as obtained by lesions of the subcortical white matter, the thalamus, or the mesencephalic reticular formation) have shown a relationship between the firing probability and the surface-positive (depth-negative) delta waves, whereas the depth-positive

waves were associated with a diminution in discharge rates (Ball et al., 1977; see Fig. 1.21 in Steriade and Buzsáki, 1990). All these field–unit relationships might lead to the assumption that the depth-positive component of delta waves reflects the inhibition of pyramidal-shaped neurons by local-circuit cells. There are numerous types of GABAergic and/or peptidergic inhibitory interneurons in various cortical areas, such as large basket cells, small to medium-sized "chandelier" neurons, "spider" or "clutch" cells, and very small bipolar interneurons (e.g. Kisvárday et al., 1983, 1993; Somogyi et al., 1983, 1984; reviewed in Jones, 1987; Steriade et al., 1990e). GABAergic interneurons open chloride channels in pyramidal-type neurons and would then produce an extracellular current flow responsible for the depth-positivity of delta waves. Such a correlation would imply maximal firing of putative inhibitory interneurons during the depth-positive delta waves. However, this has not been found. It was then suggested (Steriade and Buzsáki, 1990; Steriade et al., 1990b) that, far from resulting exclusively from IPSPs, EEG delta waves are rather generated by summation of long-lasting afterhyperpolarizations (AHPs) produced by a variety of potassium currents in deeply lying pyramidal neurons.

Intracellular Analyses of the Thalamic Delta Rhythm

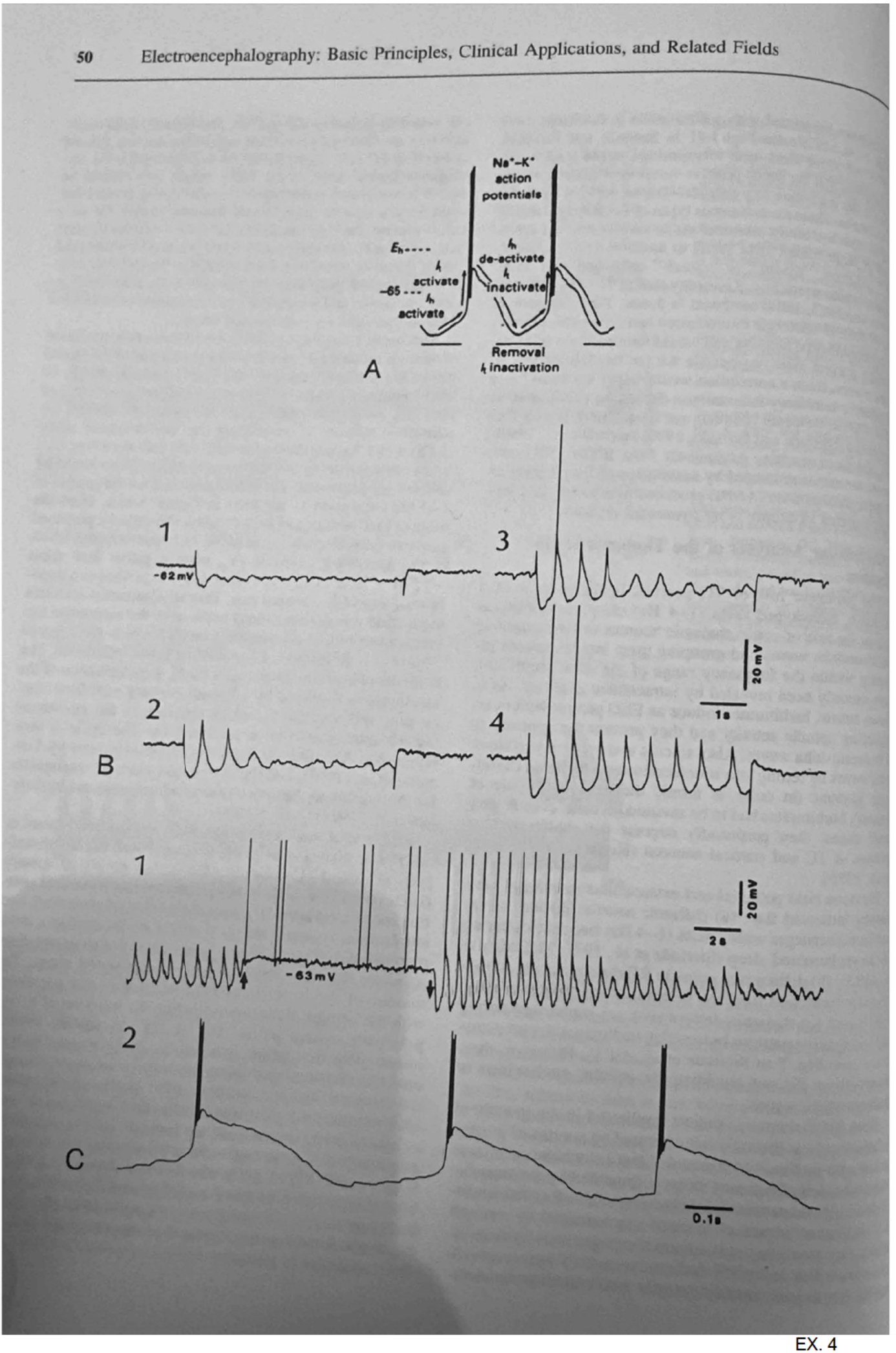
The prevalent role of TC neurons in the genesis of a clocklike, stereotyped delta (1–4 Hz) sleep oscillation, as well as the role of corticothalamic neurons in synchronizing thalamic delta waves and grouping them into sequences recurring within the frequency range of the slow oscillation, have recently been revealed by intracellular analyses. As is shown below, barbiturates induce an EEG picture with overwhelming spindle activity and they prevent the appearance of thalamic delta waves. Also, since it was necessary to block delta waves by setting into action brainstem–thalamic cholinergic systems (in order to mimic the behavioral state of arousal), barbiturates had to be avoided because, even in very small doses, they profoundly depress the cholinergic responses of TC and cortical neurons (Krnjevic, 1974; Eysel et al., 1986).

Previous field potential and extracellular recordings have already indicated that: (a) thalamic neurons display waves and unit discharges within delta (1–4 Hz) frequencies during EEG-synchronized sleep (Steriade et al., 1971; McCarley et al., 1983); (b) delta waves occur in RE thalamic nucleus even after disconnection from the cerebral cortex (Steriade et al., 1987); and (c) thalamic delta waves are suppressed during EEG-activation patterns induced by midbrain reticular stimulation (see Fig. 7 in Steriade et al., 1971). However, these observations did not elucidate the cellular mechanisms of thalamic delta waves.

That the thalamus is indeed implicated in the genesis of delta waves was recently demonstrated by a series of *in vitro* and *in vivo* studies, which revealed that a clocklike oscillation within the delta frequency range is generated by the interplay of two intrinsic currents of thalamocortical cells. This intrinsic oscillation is potentiated and synchronized by network operations involving corticothalamic projections, with an intermediate link in the RE thalamic nucleus. *Whereas spindle oscillation is generated by synaptic interactions in the thalamic networks including RE nucleus, the thalamic delta oscillation is an intrinsic oscillation depending on two inward currents of TC cells.* Nonetheless, to be expressed at the macrophysiological level of the EEG, single cells should be united into neuronal ensembles by synchronizing devices that must have access to many dorsal thalamic nuclei. On an a priori ground, the only candidate for such a synchronization process is the RE thalamic nucleus that projects to widespread dorsal thalamic territories. Data related to the intrinsic electrophysiological properties of thalamic cells implicated in delta oscillation and to network synchronization of individual thalamic neurons are summarized below.

McCormick and Pape (1990a) found that a subpopulation of neurons recorded *in vitro* from the dorsal part of the lateral geniculate thalamic nucleus displayed rhythmic bursts of high-frequency spikes with an interburst frequency of 1–2 Hz. This oscillation results from the interplay between the transient calcium, I_t, underlying the low-threshold spike (LTS) and a hyperpolarization-activated cation current (I_h). I_h is a noninactivating inward (anomalous) rectifier carried by sodium and potassium. The model proposed for the genesis of 1–2 Hz oscillation is depicted in Figure 3.18A, from the work of McCormick and Pape (1990a), showing the proposed currents mediating the oscillation. At hyperpolarized levels of the membrane potential (V_m more negative than about −65 or −70 mV), I_h is activated and is expressed as a depolarizing sag of V_m toward rest. This depolarization activates the I_t (that was de-inactivated because of the membrane hyperpolarization), thus underlying an LTS which gives rise to a burst of high-frequency fast sodium action potentials. The latter depolarization de-activates the I_h. Repolarization of the membrane is followed by a hyperpolarizing overshoot that, in turn, activates the I_h, which depolarizes the membrane toward another calcium-dependent LTS. The cycle is then repeatable. A similar rhythm was described *in vitro* by Leresche et al. (1991), and the two inward currents responsible for this oscillatory behavior have also been analyzed by Soltesz et al. (1991).

Our *in vivo* studies (Steriade et al., 1991a; Curró Dossi et al., 1992a; Nuñez et al., 1992b) have shown that antidromically identified TC cells recorded from a variety of sensory (lateral geniculate and ventral posterior), motor (ventral anterior and ventral lateral), associational (lateral posterior), and intralaminar (central lateral) thalamic nuclei display a delta rhythm induced by imposed hyperpolarization to values characteristic for late stages of EEG-synchronized sleep. The number of cycles in the elicited oscillation was correlated with the voltage deflection following the injection of hyperpolarizing current pulses (Fig. 3.18B). Moreover, *spontaneous* delta oscillation (without injecting current pulses) could be obtained by ablating the cortical areas projecting to the recorded thalamic nucleus. This deafferentation procedure removed the powerful depolarizing impingement from corticothalamic neurons and set thalamic cells at a more hyperpolarized V_m (around −70 mV) where delta oscillation is generated (Fig. 3.18C). The fact that rhythmic LTSs occurred in isolation (without superimposed fast action potentials that could have synaptically engaged other elements in the thalamic and cortical networks) supported the idea that this oscillation is intrinsic.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Na⁺–K⁺ action potentials
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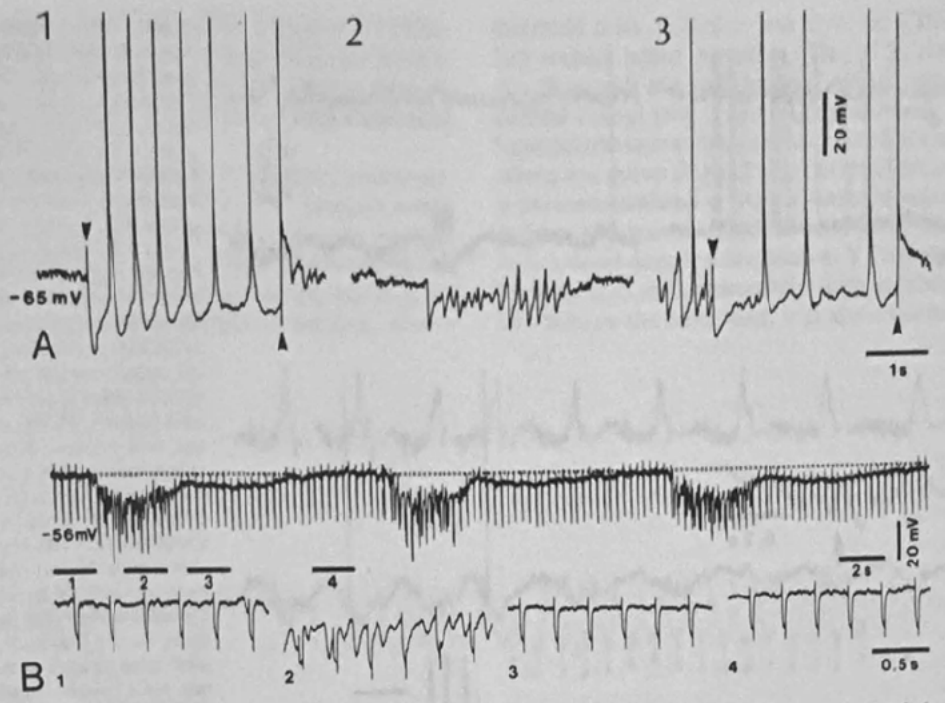


Figure 3.19. Incompatibility, at the single-cell level, between spindle and delta oscillations. Intracellular recordings of two (**A** and **B**) ventroanterior thalamocortical cells in cerveau isolé cats. **A.** 1: Hyperpolarizing current pulse (1.5 nA) generated delta oscillation at 2.5 Hz. 2: Spindle sequence occurring spontaneously. 3: Spindle sequence beginning before the hyperpolarizing current pulse (same parameters as in 1) blocked delta oscillation. Note smaller voltage deflection evoked by hyperpolarizing current pulse during spindles. **B.** Change in membrane conductance during spindle sequences. Spontaneous spindle sequences show that, after the rhythmic IPSPs of spindles, the membrane potential remains hyperpolarized for 3–5 seconds. Short hyperpolarizing current pulses (0.8 nA) were injected to measure the membrane conductance. Epochs 1–4 are expanded below. Note increased conductance during spindles and, less marked, during the hyperpolarization following spindles, compared to control interspindle lulls. (Modified from Nuñez, A., Curró Dossi, R., Contreras, D., and Steriade, M. 1992b. Intracellular evidence for incompatibility between spindle and delta oscillations in thalamocortical neurons of cat. Neuroscience 48:75–85.)

Several other features characterize the intrinsic thalamic delta oscillation, as revealed in our *in vivo* experiments.

(a) This oscillation is sensitive to barbiturates; even very low doses of short-acting barbiturates are effective in blocking it (Curró Dossi et al., 1992a). This effect is due to an increased conductance of TC cells produced by barbiturates, as already reported in *in vitro* experiments (Sykes and Thomson, 1989). The increased conductance prevents the interplay between the two inward currents responsible for the delta genesis. The sensitivity to barbiturates also explains why delta oscillation was not found in previous experiments on oscillatory properties of TC cells conducted under barbiturate anesthesia.

(b) Delta oscillation is also blocked by spindle sequences, even those that occur in cerveau isolé preparations in the absence of barbiturates (Nuñez et al., 1992b) (Fig. 3.19A). The proposed mechanism of delta occlusion by spindles is the increased membrane conductance that is seen during and a few seconds after a spindle sequence (Fig 3.19B). The hyperpolarization of TC cells outlasting the spindle sequence

Figure 3.18. Delta oscillations in thalamocortical cells result from the interplay between two intrinsic currents, I_h and I_t. **A.** Proposed model for interaction between these intrinsic currents. Activation of the low-threshold calcium current, I_t, depolarizes the membrane toward threshold for a burst of sodium-dependent fast action potentials. The depolarization inactivates the portion of I_h that was active immediately before the calcium spike. Repolarization of the membrane due to I_t inactivation is followed by a hyperpolarizing overshoot, owing to the reduced depolarizing effect of I_h. The hyperpolarization in turn deinactivates I_t and activates I_h, which depolarizes the membrane toward threshold for another calcium spike. **B.** Delta oscillation of cat ventral lateral thalamocortical cell triggered by hyperpolarizing current pulses (0.7 nA in *1*, 1 nA in 2, 1.1 nA in *3*, and 1.2 nA in *4*). Note increasing number of cycles at a frequency of 1.6 Hz. **C.** Lateral posterior (LP) thalamocortical cell after decortication of areas projecting to LP nucleus. The cell oscillated spontaneously at 1.7 Hz. A 0.5-nA depolarizing current (between arrows) prevented the oscillation, and its removal set the cell back in the oscillatory mode. Three cycles after removal of depolarizing current in 1 are expanded in 2 to show high-frequency bursts crowning the low-threshold calcium spike. (*A* modified from McCormick, D.A., and Pape, H.-C. 1990a. Properties of a hyperpolarized-activated cation current and its role in rhythmic oscillation in thalamic relay neurons. J. Physiol. (Lond.) 431: 291–318. *B* and *C* modified from Steriade, M., Curró Dossi, R., and Nuñez, A. 1991a. Network modulation of a slow intrinsic oscillation of cat thalamocortical neurons implicated in sleep delta waves; cortically induced synchronization and brainstem cholinergic suppression. J. Neurosci. 11:3200–3217.)

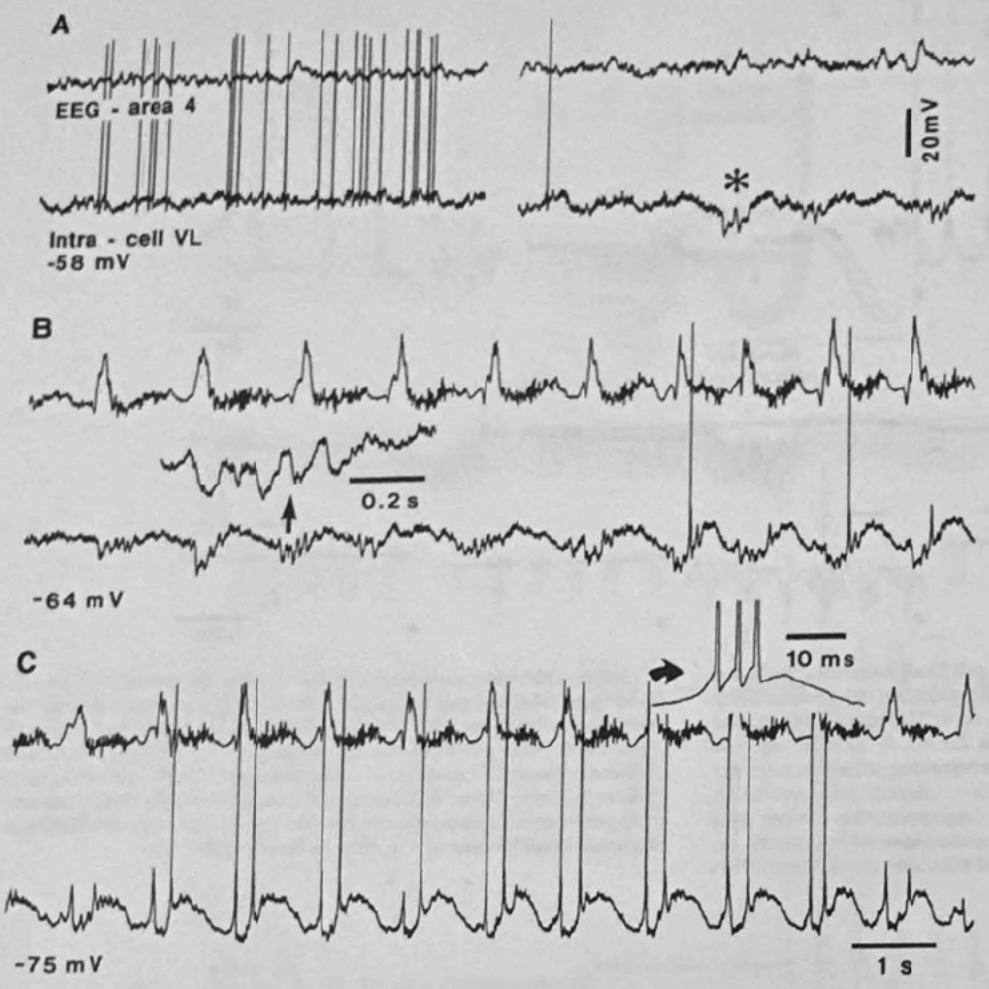


Figure 3.20. The dependency of [illegible] tion from spindle to delta oscillation [illegible] membrane potential of thalamocortical (TC) neurons in a cat under ketamine-[illegible] lazine anesthesia. Intracellular recording of a neuron from the ventral lateral nucleus simultaneously with surface-EEG from cortical area 4. **A.** *Left:* Tonic firing during activated EEG pattern; *right:* appearance of membrane potential oscillations in association with slight changes in EEG activity. Asterisk marks the first hyperpolarizing wave in sequence in association with the initial signs of EEG synchronization). **B.** Hyperpolarizing spindle oscillations (8–10 Hz) are grouped within sequences recurring with a slow rhythm (~0.8 Hz), in close time-relation with the slow cortical EEG rhythm. One spindle sequence is expanded above (arrow). **C:** A hyperpolarizing current pulse (−0.2 nA) transformed spindles into delta potentials (3 to 4 Hz), grouped by the same slow cortical rhythm (~0.8 Hz). One spike burst crowning the low-threshold spike is expanded above. (From Steriade, M., Contreras, D., and Amzica, F. 1994. Synchronized sleep oscillations and their paroxysmal developments. Trends Neurosci. 17: 199–208.)

is ascribed to the long-lasting, tonic spike barrage of RE thalamic cells that follows the spindle-related spike bursts in this GABAergic cell-type (Steriade et al., 1986). The increased membrane conductance during this prolonged hyperpolarization probably unbalances the interplay between I_t and I_t. This incompatibility between spindles and delta waves occurs at the singular cellular level. The presence of spindles at the EEG level (though they are greatly reduced) during late sleep stages when they may coexist with delta waves is due to the fact that some neuronal pools are still at the V_m where spindles are prevalent (see below, point c).

(c) Spindles and delta oscillations appear at different V_m of TC cells. At the resting V_m (around −60 mV), thalamic cells display spontaneous or cortically elicited spindle oscillation, whereas at V_m more negative than −65 or −70 mV spindles progressively decrease in amplitude (due to the partial reversal of IPSPs that compose the cyclic hyperpolarizations in thalamocortical neurons) and the oscillations are within the delta frequency range (Fig. 3.20). Since delta oscillations, prevailing during late sleep stages, occur spontaneously or are triggered by cortical volleys at more negative V_m than spindles that characterize the early sleep stage, we have postulated a progressive hyperpolarization of thalamocortical cells with the deepening of sleep with EEG synchronization (Steriade et al., 1991a). This is attributable to the progressive decrease in firing rates, during slow-wave sleep, of corticothalamic cells (Steriade, 1978) and thalamically projecting neurons located in the midbrain reticular formation (Steriade et al., 1982), mesopontine cholinergic nuclei (Steriade et al., 1990a), and monoaminergic nuclei (Steriade and McCarley, 1990). The transmitters of all these neuronal types produce a depolarization of TC cells (McCormick and Prince, 1987, 1988; McCormick, 1990). That the partial removal of the depolarizing impingement exerted by corticothalamic cells is also effective in producing the V_m hyperpolarization required for setting thalamic neurons in the delta oscillatory mode (Curró Dossi et al., 1992a) is in keeping with Buzsáki's (1991) results, which showed that injections of NMDA blockers lead to a reduction in frequency of thalamic oscillations from 8 to 2 Hz. It is known from human and animal studies that spindle and delta prevail during different sleep stages and that these two rhythms reciprocally oscillate within EEG

synchronized sleep (Uchida et al., 1991; Lancel et al., 1992). The intracellular data reported above provided the mechanisms accounting for this relative incompatibility between the two major sleep rhythms, mainly due to their differential voltage dependency.

(d) Cortical volleys potentiate delta oscillation in thalamocortical cells (Steriade et al., 1991a). This synaptic action indicates that delta oscillation, resulting from the intrinsic properties of thalamic cells, is powerfully modulated by network operations. The facilitatory process is expressed by the transformation, as an effect of cortical stimulation, of subthreshold delta oscillation into rhythmic LTSs crowned by fast sodium action potentials. The LTSs may persist for 10–20 seconds as a self-sustained activity, after cessation of cortical volleys (Fig. 3.21A). Cortical stimuli also revive a hyperpolarization-activated delta oscillation when it dampens after a few cycles (Fig. 3.21B). The question may arise: how is the corticothalamic activity involved in maintaining a high level of activation in thalamic cells (since cortical ablation or functional disconnection leads to V_m hyperpolarization of thalamic cells and, consequently, delta oscillations; see point c) while, on the other hand, it is also capable to potentiate

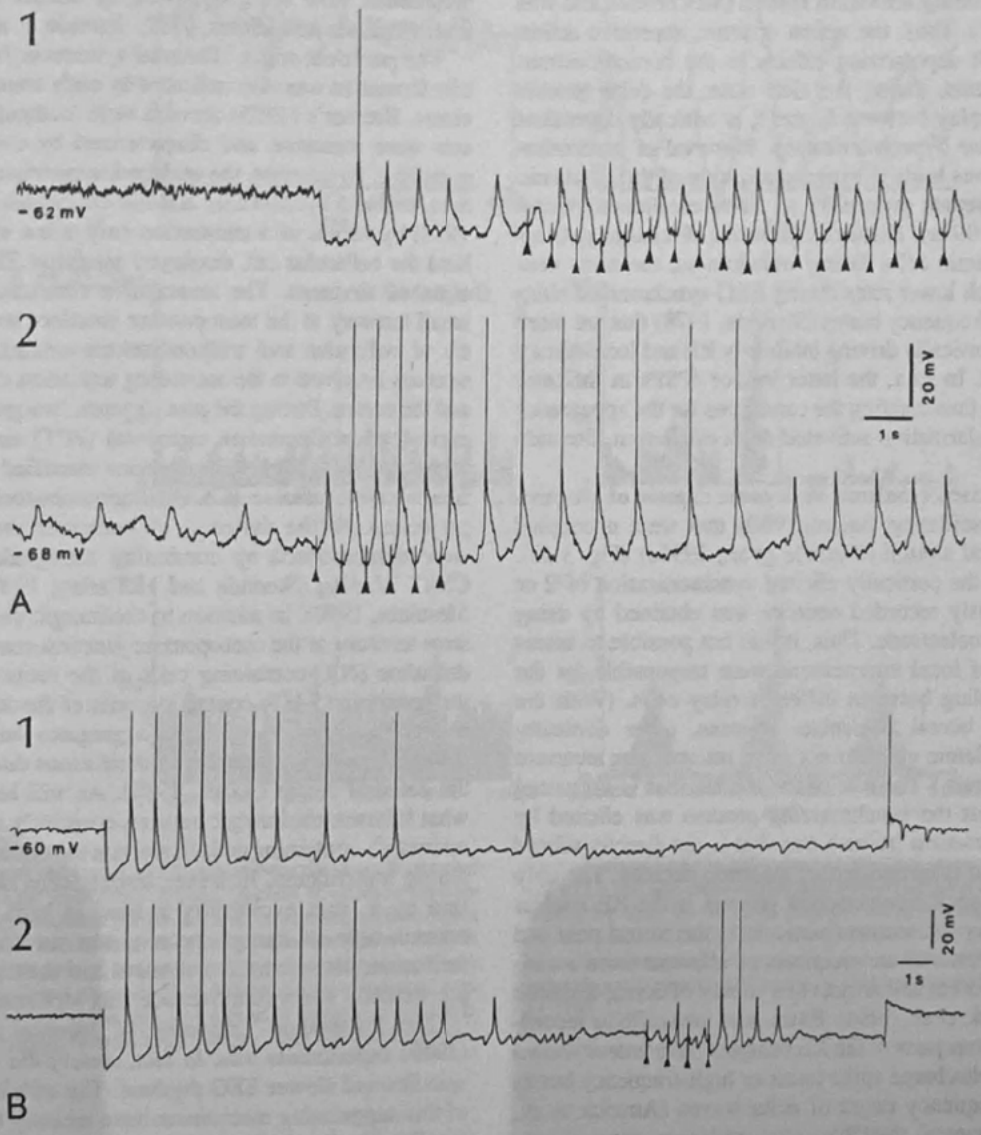


Figure 3.21. Cortical potentiation of thalamic delta oscillation. Intracellular recordings in cats under urethane anesthesia. **A.** Ventral lateral thalamocortical cell. *1.* Hyperpolarization (0.8 nA) bringing the V_m from −62 to −78 mV induced a 2-Hz oscillation. When dampening of oscillation was imminent, cortical stimuli at 2 Hz evoked low-threshold spikes (LTSs). *2.* At V_m of −68 mV, a subthreshold oscillation appeared; six cortical stimuli (same parameters as in *1*) induced LTSs and, after cessation of stimuli, a self-sustained oscillation at 1–1.5 Hz ensued for 15 seconds. **B.** Another neuron, located in the centrolateral nucleus. Hyperpolarizing current pulses (duration, 22.5 seconds, 0.9 nA in *1*, 1 nA in *2*) elicited 1.4-Hz oscillation that dampened after 6 seconds (*1*) and 9 seconds (*2*). Four shocks to the precruciate gyrus (*2*, arrowheads) restarted the dampened oscillation which, thereafter, continued in a self-sustained manner. (Modified from Steriade, M., Curró Dossi, R., and Nuñez, A. 1991a. Network modulation of a slow intrinsic oscillation of cat thalamocortical neurons implicated in sleep delta waves; cortically induced synchronization and brainstem cholinergic suppression. J. Neurosci. 11:3200–3217.)

delta oscillation formed by alternating LTS-AHP sequences on a background of increased hyperpolarization? The answer is that the influence of cortical cells on the thalamus varies with the behavioral state. Tonic, high-discharge firing rates of corticothalamic cells during waking (Steriade, 1978) exert a depolarizing impingement on thalamic cells through the release of excitatory amino acids acting on non-NMDA, NMDA, as well as metabotropic glutamate receptors (McCormick and von Krosigk, 1992). Particularly, the prolonged excitation of thalamic relay cells resulting from the reduction of a "leak" potassium conductance through the activation of glutamatergic metabotropic receptors is capable of maintaining the activation patterns of TC cells, thus acting as a true descending activation system (McCormick and von Krosigk, 1992). Thus, the action of tonic, repetitive action potentials with depolarizing effects in the corticothalamic pathway prevents, during the alert state, the delta genesis since the interplay between I_h and I_t is critically dependent upon membrane hyperpolarization. Removal of corticothalamic projections leads to hyperpolarization of their thalamic targets and greater propensity to delta oscillation (Curró Dossi et al., 1992a). Distinctly from the discharge patterns of corticothalamic cells during wakefulness, the same neurons have much lower rates during EEG-synchronized sleep and fire high-frequency bursts (Steriade, 1978) that are more effective in phasically driving inhibitory RE and local-circuit thalamic cells. In turn, the latter induce IPSPs in thalamic relay neurons, thus creating the conditions for the appearance of the hyperpolarization-activated delta oscillation (Steriade et al., 1991a).

(e) Lastly, corticothalamic volleys are capable of synchronizing delta-oscillating thalamic cells that were uncoupled prior to cortical stimuli (Steriade et al., 1991a) (Fig. 3.22). In this study, the cortically elicited synchronization of 2 or 3 simultaneously recorded neurons was obtained by using the same microelectrode. Thus, it was not possible to assess whether RE of local interneurons were responsible for the synaptic coupling between different relay cells. (With the exception of lateral geniculate neurons, other cortically projecting thalamic cells do not have intranuclear recurrent axonal collaterals.) The role of the RE nucleus is suggested by the fact that the synchronizing process was elicited by stimulating a cortical (motor) area that is not directly related to the recorded (lateroposterior) thalamic nucleus. The only candidate for such a conjunction process is the RE nuclear complex. Many RE sectors (particularly the rostral pole and rostrolateral districts) are recipients of afferents from a variety of cortical areas and project to a variety of dorsal thalamic nuclei (Steriade et al., 1984). Extra- and intracellular recordings from various parts of the RE complex have indeed shown that RE cells discharge spike trains or high-frequency bursts within the frequency range of delta waves (Amzica et al., 1992; Steriade et al., 1993b).

Fast Rhythms (20–50 Hz) during Diffuse Arousal and Focused Attention

The brain substrate of EEG activation upon arousal has begun to be understood since the pioneering work of Moruzzi and Magoun (1949). They stimulated different foci of the brainstem reticular core in the deeply anesthetized (...osed) cat and elicited the transformation of high-volta... frequency cortical EEG waves into low-voltage, fast r... reminiscent of those occurring upon natural awaken... cortical effect was thought to be mediated, at least ... by the diffuse thalamic projecting system. Although th... response was obtained from many brainstem foci b... the medulla and the mesencephalon (whence the no... unspecific arousal systems), the most effective point ... cated in the rostral reticular core. Both the rostral bra... reticular origin of at least one category of arousing n... and their activating effects upon the cerebral cortex t... a synaptic linkage in thalamic nuclei with widespread c... projections have been supported by studies at the c... level (Steriade and Glenn, 1982; Steriade et al., 1982...

The prevalent origin of arousal systems in the rostral ... ular formation was also indicated by early transection e... ments. Bremer's (1935) cerveau isolé (collicular-transe... cats were comatose and characterized by continuous ... spindling. By contrast, the midpontine pretrigeminal pre... tion, realized by Moruzzi and his colleagues (Batini e... 1958) by means of a transection only a few millimeter... hind the collicular cut, displayed persistent EEG and o... signs of alertness. The inescapable conclusion was th... small territory at the mesopontine junction, between the ... els of collicular and midpontine transections, contains ... neurons involved in the ascending activation of the thala... and the cortex. During the past 15 years, two groups of cho... ergic (pedunculopontine tegmental [PPT] and laterodo... tegmental [LDT]) nuclei have been identified by using c... line acetyltransferase (ChAT) immunohistochemistry. T... projections to the thalamus of brainstem cholinergic c... were demonstrated by combining retrograde tracers w... ChAT labeling (Steriade and McCarley, 1990; Wainer a... Mesulam, 1990). In addition to cholinergic nuclei, the bra... stem territory at the mesopontine junction contains the no... drenaline (NA)-containing cells of the locus coeruleus a... the serotonin (5-HT)-containing cells of the dorsal raphe ... cleus. These monoaminergic aggregates have much l... dense thalamic projections, but their axons directly innerv... the cerebral cortex (Saper, 1987). As will become clear ... what follows, cholinergic neurons conjointly act with mo... aminergic-containing cells to activate the thalamus and cor... during wakefulness. However, during REM sleep, charact... ized by a brain excitability at least as high as that duri... arousal, only cholinergic neurons can account for forebr... activation, because locus coeruleus and dorsal raphe neuro... are virtually silent (see Steriade and McCarley, 1990).

The "activation" response of Moruzzi and Magoun ... (1949) experiments was, in fact, merely the suppression ... spindles and slower EEG rhythms. The cellular mechanis... of this suppressing mechanism have recently been disclos... (a) The blockage of spindle oscillations takes place at t... very site of their genesis, the RE thalamic nucleus. Brainste... cholinergic neurons induce a hyperpolarization of RE ce... by increasing a potassium conductance and, thus, they c... off the depolarizing envelope of spindle oscillations in t... neurons (McCormick and Prince, 1986; Hu et al., 198... Other activating neuromodulators, 5-HT and NA, depolari... RE neurons (McCormick and Wang, 1991) and this acti...

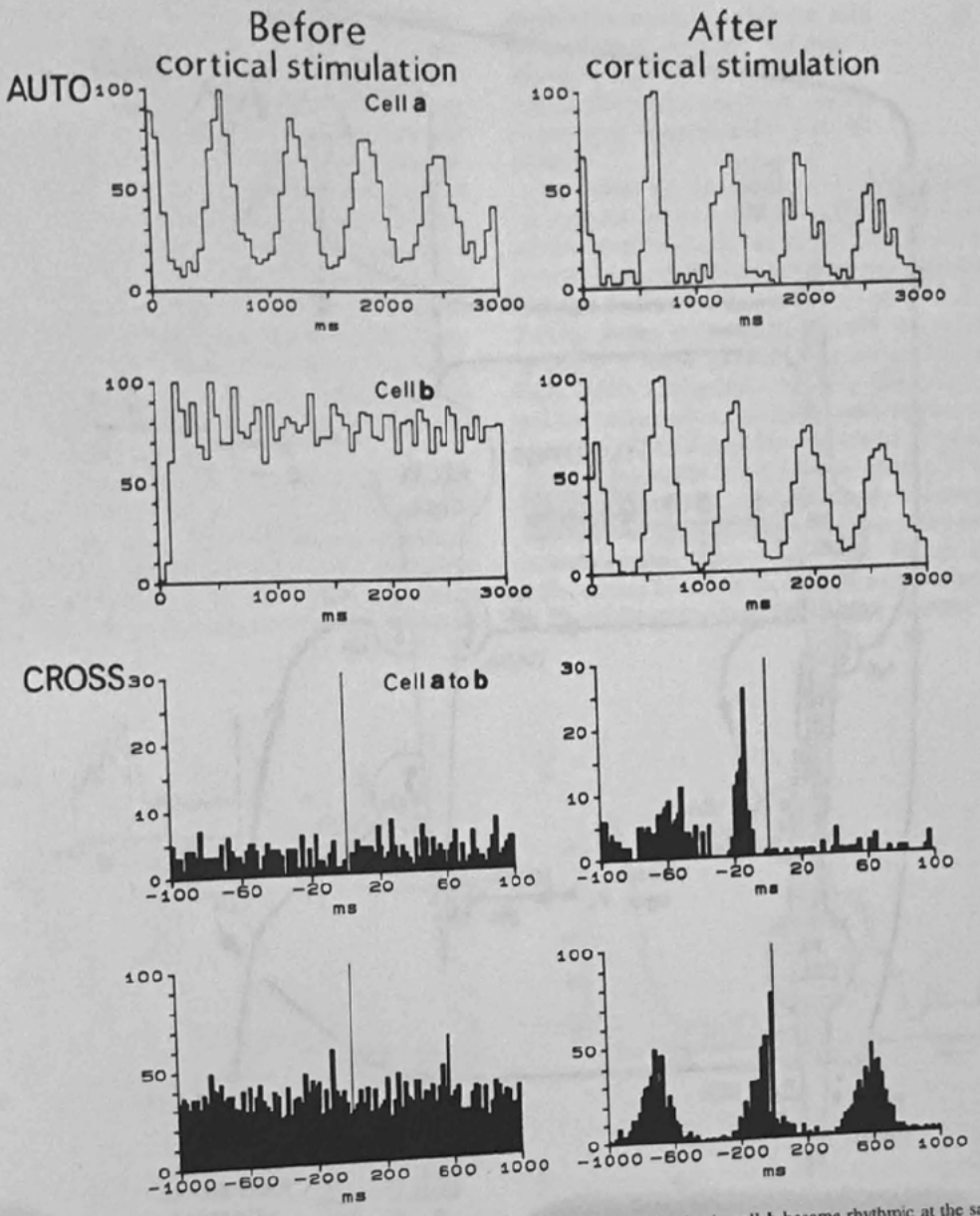


Figure 3.22. Auto- and cross-correlograms of two cells (**a** and **b**) recorded simultaneously in the ventrolateral thalamic nucleus of cat under urethane anesthesia. Four correlograms (before and after cortical stimulation) depict, from top to bottom, autocorrelogram of cell **a**, autocorrelogram of cell **b**, and the cross-correlogram of both cells (cell **b** is the reference cell) with two different bins (2 and 20 msec). Note, before cortical stimulation, delta (1.6-Hz) rhythm of cell **a**, flat contour (absence of rhythmicity) in cell **b**, and absence of coupling between these neurons. After cortical stimulation, the background noise in cell **b** became rhythmic at the same frequency as cell **a** (1.6 Hz), and cross-correlograms show that cell **a** firing preceded cell **b** firing by about 10–20 msec. (Modified from Steriade, M., Curró Dossi, R., and Nuñez, A. 1991a. Network modulation of a slow intrinsic oscillation of cat thalamocortical neurons implicated in sleep delta waves; cortically induced synchronization and brainstem cholinergic suppression. J. Neurosci. 11:3200–3217.)

promotes single-spike activity of RE neurons, much the same as arousal. However, the combined effects of ACh, 5-HT, and NA, having quite different actions on the spindle pacemaking RE neurons, are far from being understood. (b) Thalamic delta waves are blocked by cholinergic (Steriade et al., 1991a) and monoaminergic (McCormick and Pape, 1990b) neurons, which exert depolarizing actions on their thalamic targets, and thus, bring them out of the voltage range where the intrinsic, clocklike delta oscillation is generated. Cortical delta waves are blocked by the cholinergic actions of nucleus basalis neurons (Buzsáki et al., 1988). The complex action of modulatory cholinergic neurons on different types of thalamic and cortical cells is summarized in the cellular recordings and diagrams of Figure 3.23.

At the time of Moruzzi and Magoun (1949), "activation" was thought to consist of negative events (suppression of

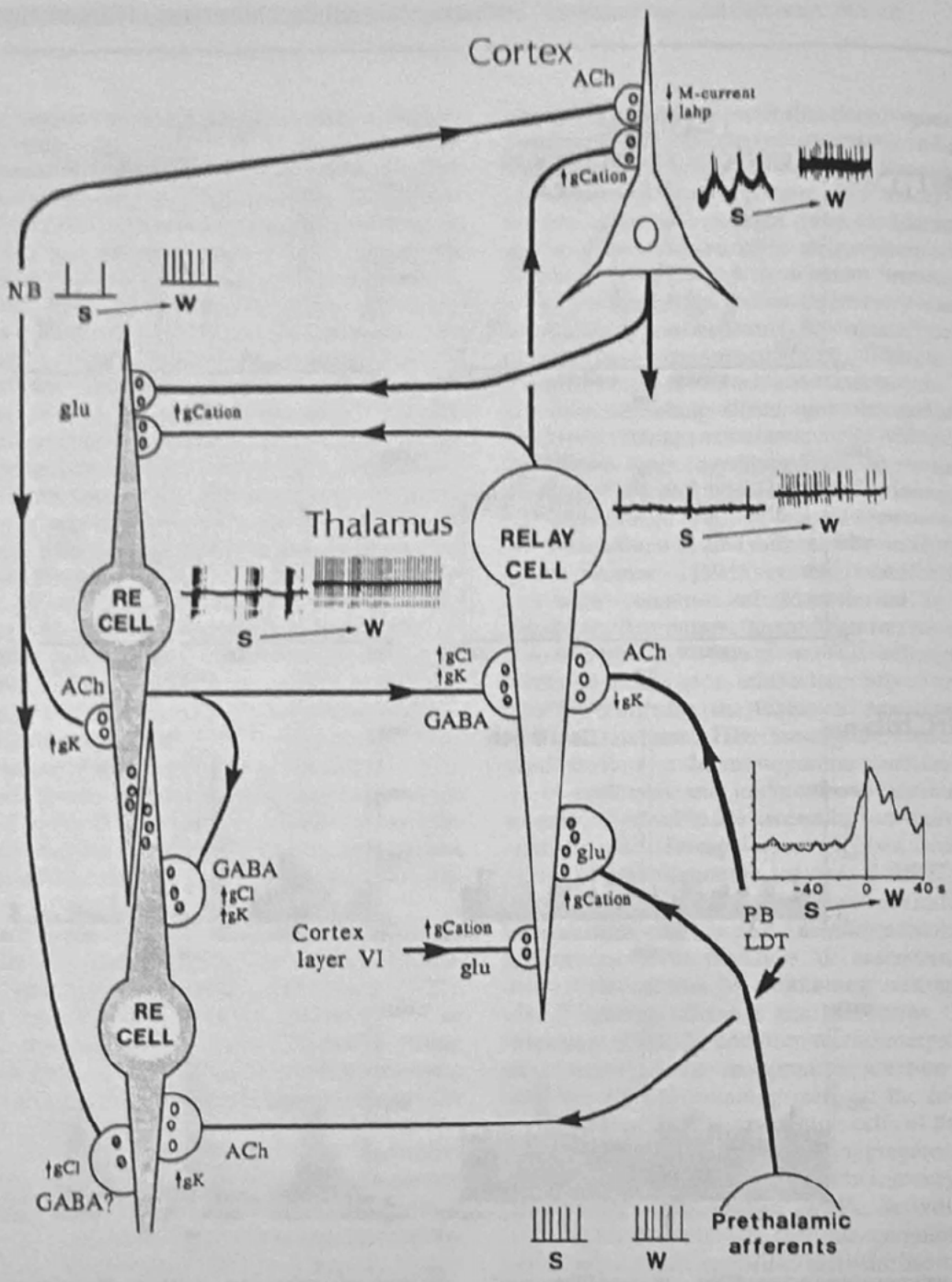


Figure 3.23. Schematic diagram of ascending cholinergic actions upon thalamocortical (relay) neurons, reticular thalamic (*RE*) GABAergic neurons, and pyramidal-shaped neurons of the cerebral cortex. Direction of axons is indicated by arrows. Acetylcholine (*ACh*) is released in the thalamus by peribrachial (*PB*) neurons of the pedunculopontine tegmental (*PPT*) nucleus and by laterodorsal tegmental (*LDT*) neurons. In the cortex, ACh is mostly released by nucleus basalis (*NB*) neurons. In relay and RE thalamic cells as well as in cortical pyramidal neurons, EEG-synchronized sleep (*S*) is characterized by rhythmic inhibitory periods and bursts of action potentials within the frequency range of spindle oscillations. These processes are associated with a decreased transfer function in thalamocortical cells. In cortical neurons, the activity evoked by prethalamic afferents is further reduced due to a relatively uninhibited M-current and slow afterhyperpolarizing current (I_{ahp}), which underlie spike frequency adaptation. Upon transition from S to waking (*W*), cholinergic PB/LDT neurons increase their rates of spontaneous firing, about 20 seconds before time 0 of W, taken as the most precocious change from EEG synchronization to desynchronization. An increased activity from S to W is also seen in NB neurons. Note that no change in firing rate from S to W is seen in neurons recorded from specific prethalamic relays. Increased activity of cholinergic PB/LDT and NB cells di rhythm generation in the thalamus by depolarizing relay neurons, th a decrease in potassium conductance, g_K. As well, cortical pyramida are activated through a decrease in M-current and I_{ahp}. There is, in ad an excitatory action on cortical pyramidal cells by noncholinergic matergic) thalamocortical cells (increase in cationic permeability). Be the hyperpolarization of RE cells by cholinergic PB/LDT cells as w by NB cholinergic and GABAergic cells underlies the blockage of s generation in the pacemaking RE neurons. Although PB/LDT choli afferents hyperpolarize RE cells by an increase in g_K, these neuro brought upon arousal toward single-spike firing, with increased dis rates, because they are subject to excitatory influences from both rel cortico-RE neurons using excitatory amino acids (*glu*). Schemes of ch in ionic conductances of thalamic and cortical neurons are modifie McCormick (1990) based on *in vitro* data of McCormick and Prince 1987). Data related to the S-W behavior of various types of depicted n are from chronic experiments on PB/LDT (Steriade et al., 1990a), RE mic (Steriade et al., 1986), and thalamocortical and cortical pyramid (Steriade et al., 1990c).

EEG-synchronized waves), but no real sign of activated processes was observed, as their results mainly reported the flattening of the EEG following brainstem reticular formation. Only a decade later, evidence of increased excitability was provided. Cortical field potentials evoked by stimulation of prethalamic fibers (for example, the optic tract) were enhanced during midbrain reticular stimulation (Bremer and Stoupel, 1959; Dumont and Dell, 1960). In parallel studies, photically evoked field potentials recorded from the lateral geniculate thalamic nucleus were increased during brainstem reticular-induced arousal, without any sign of enhanced responses simultaneously recorded from the optic tract (Steriade and Demetrescu, 1960). Thus, the notion of activation was strengthened by data showing an increased synaptic excitability of both thalamic and cortical neurons during mesencephalic reticular stimulation.

The first demonstration that the EEG-activated response to brainstem reticular stimulation is not only the blockage of spindles and slow waves, but also includes the appearance of peculiar fast rhythms characterizing arousal, was made by Bremer and colleagues (1960). They reported that a flattening of the cortical EEG is not the constant effect of brainstem stimulation. Instead, a clear-cut enhancement in amplitude of spontaneous rhythms and their regular acceleration to 40–45 Hz (*accélération synchronisatrice*) appeared on the cortical EEG of the *encéphale isolé* (bulbospinal transected) preparation, simultaneously with the ocular syndrome of arousal.

Since then, a series of studies in various cortical areas have reported the presence of 20–40 Hz waves during different conditions of increased alertness. The fast rhythm was observed in the occipital cortex while the dog paid intense attention to a visual stimulus (Lopes da Silva et al., 1970; Fig. 3.24A), during accurate performance of a conditioned response to a visual stimulus in monkey (Freeman and Van Dijk, 1988), during tasks requiring fine finger movements and focused attention in monkey motor cortical cells (Murthy and Fetz, 1992), during focused arousal prior to the performance of a complex task in humans (Sheer, 1984), and during behavioral immobility associated with an enhanced level of vigilance while the cat was watching a visible but unseizable mouse (Rougeul-Buser et al., 1983; Bouyer et al., 1987). Other studies have described stimulus-dependent oscillations at 25–45 Hz of the focal EEG and/or neuronal firing in the

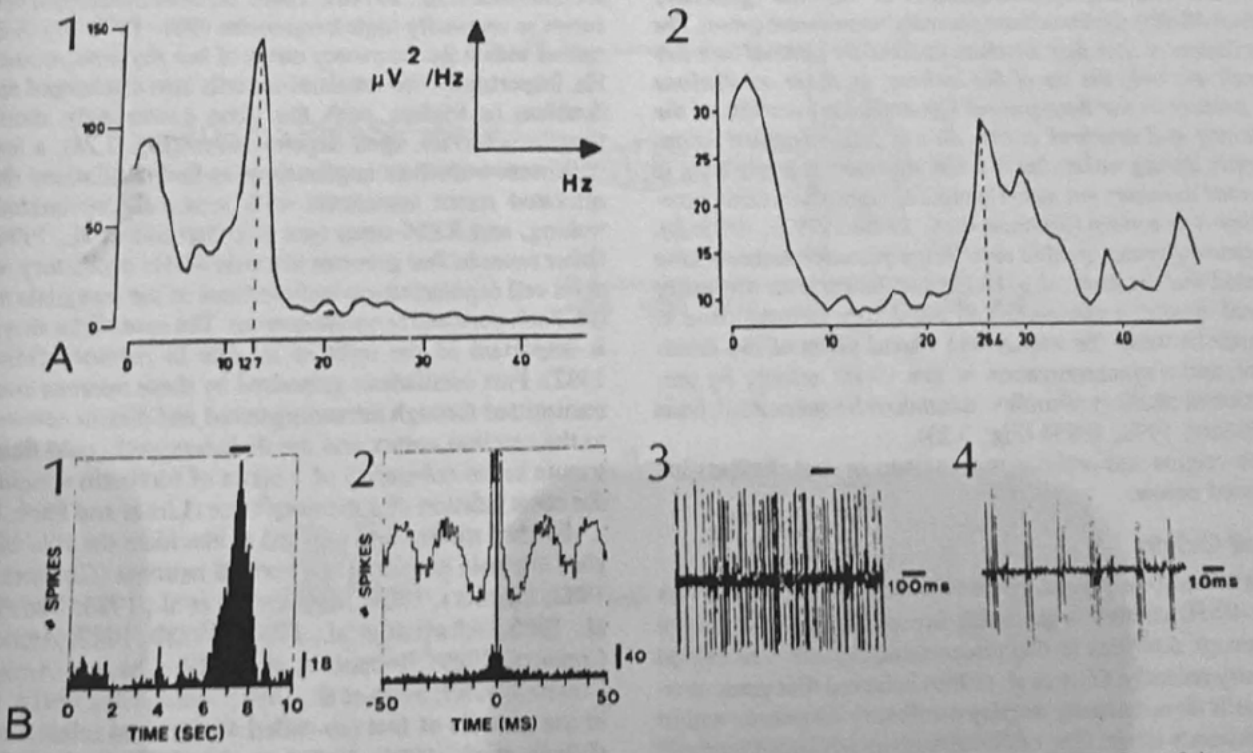


Figure 3.24. Induced beta (25–40 Hz) oscillations in visual cortex. **A.** Power spectra calculated from EEG signals recorded from the surface of the occipital cortex in the dog. The spectra in 1 were obtained while the awake animal kept its eye closed, and those in 2 when the animal paid intense attention to a visual object. Note that cortical activity had different dominant frequency components: alpha activity dominates in 1 and beta activity (peak around 26 Hz) dominates in 2. **B.** The firing pattern of an oscillatory standard-complex cell of cat. 1. Poststimulus time-histogram of the neuronal spike train recorded over 10 trials. The response is selective for the second direction of stimulus movements. 2. Autocorrelograms. Filled and unfilled bars display the correlograms computed for the first and second direction of stimulus movement, respectively. 3. Plot of the spike train recorded during a single presentation to the second direction of stimulus movement. 4. High-resolution plot of the same data shown in 3. (*A*, modified from Lopes da Silva, F.H., Van Rotterdam, A., Storm van Leeuwen, W., and Tielen, A.M. 1970. Dynamic characteristics of visual evoked potentials in the dog. II. Beta frequency selectivity in evoked potentials and background activity. Electroencephalogr. Clin. Neurophysiol. 29:260–268. *B*, modified from Gray, C.M., Engel, A.K., König, P., and Singer, W. 1990. Stimulus-dependent neuronal oscillations in cat visual cortex: receptive field properties and feature dependence. Eur. J. Neurosci. 2:607–619.)

olfactory system (Freeman, 1975) and visual cortex (Eckhorn et al., 1988; Gray and Singer, 1989; Engel et al., 1990; Gray et al., 1989, 1990) (Fig. 3.24B). It was also reported that brainstem reticular stimulation selectively enhances the 80-Hz oscillatory waves composing the afterdischarge of the flash-evoked response in the visual cortex (Steriade et al., 1968) and facilitates the coherency of 40-Hz responses in visual cortical neurons (Singer, 1990b). The possible significance of this rhythm resides in the fact that, in addition to the spatial mapping that allows a limited number of representations, a temporal component is brought about by synchronized oscillatory (around 40 Hz) responses across spatially separate cortical columns (Eckhorn et al., 1988; Gray et al., 1989). The conjunction of spatial and temporal factors is the basis of functionally coherent cell assemblies, distinguished by the phase and the frequency of their synchronous oscillatory activity (Singer, 1990a). The cell assemblies link spatially distributed elements and may be the bases for global and coherent properties of patterns, a prerequisite for scene segmentation and figure–ground distinction (Singer, 1990b). This "feature binding" and pattern recognition function was extended by data showing the role of 40-Hz oscillations in the mediation of higher-order motor functions (Murthy and Fetz, 1992).

The site of origin, underlying intrinsic membrane properties, and network synchronization of the fast (generally termed 40-Hz) rhythms have recently been investigated. *The conclusion is that fast rhythms elicited by optimal sensory stimuli are only the tip of the iceberg, as these oscillations are present in the background (spontaneous) activity of the thalamus and cerebral cortex during cells' depolarization, not only during wakefulness when information processing is expected to occur, but also during deep anesthesia and natural slow-wave sleep* (Steriade et al., 1991b, 1993c, 1996a,b). Magnetoencephalographic recordings in awake humans have revealed the presence of a 40-Hz oscillation over the entire cortical mantle, a phase-shift of oscillatory activity close to 12 msec between the rostral and caudal poles of the hemisphere, and a synchronization of this 40-Hz activity by presentation of auditory stimuli with random frequencies (Llinás and Ribary, 1992, 1993) (Fig. 3.25).

The origins and cellular mechanisms of fast rhythms are discussed below.

Site of Origin

While the role played by cortical neurons in the genesis of the 40-Hz rhythm is generally accepted, the participation of resonant activities in thalamocortical circuits was denied until very recently. Gray et al. (1989) believed that geniculostriate cells do not usually display oscillatory responses within this frequency range. One of the arguments advanced by those authors against the mediation of 40-Hz rhythms by TC cells was that the collaterals of lateral geniculate neurons do not span sufficiently long distances to account for synchronized oscillations in columns of the visual cortex separated by up to 7 mm. More recent studies have demonstrated that 40-Hz rhythmic activities appear in intracellularly recorded, antidromically identified TC cells (Steriade et al., 1991b, 1993c, 1996b). Other subcortical structures also have the intrinsic and network properties that could generate the fast rhythm not only at the single-cell, but also at the population. The point is that the coherency between different 40-Hz-generating brain levels can be accomplished by different types of synaptic linkages within the cortex, mus, or other structures, and by reciprocal projection tween distant brain structures (see below).

Intrinsic Membrane Properties and Network Synchronization

Llinás and collaborators (1991) have reported that th Hz rhythm can be generated by intrinsic membrane prop of sparsely spinous interneurons recorded *in vitro* from IV of guinea pig frontal cortex. It was found that these generate narrow-frequency (35–45 Hz) oscillations upo polarization of the membrane potential and that these os tions are generated by a voltage-dependent, persistent so current, with the involvement of a delayed rectifier. Su voltage-dependent fast rhythms have been intracellu recorded in identified callosal neurons (Nuñez et al., 19 a result which would explain the coherence of 40-Hz rh in cortical areas of both hemispheres (see below). That TC cells do also display the same oscillatory proper shown in Figure 3.26 from *in vivo* experiments, depict special class of rostral intralaminar nucleus, projecting high conduction velocities (40–50 m/sec) to association tex (Steriade et al., 1991b). These TC cells discharged sp bursts at unusually high frequencies (900–1000 Hz) tha curred within the frequency range of fast rhythms, aroun Hz. Importantly, the intralaminar cells also discharged sp doublets or triplets, with the same exceedingly short terspike intervals, upon depolarization (Fig. 3.26), a fea consistent with their implications in fast oscillations du activated states associated with neuronal depolarizati waking, and REM sleep (see also Steriade et al., 1996 Other neurons that generate intrinsic 40-Hz oscillatory w upon cell depolarization include those of the amygdala nu (D. Paré, personal communication). The case of the amyg is important in the light of its role in memory (Mish 1982). Fast oscillations generated by these neurons coul transmitted through intraamygdaloid and distant connect to the cerebral cortex and the thalamus, and could thus c tribute to the coherency of a series of forebrain structure the consolidation of a memory trace (Llinás and Paré, 19

Further studies are required to elucidate the role of c plex intrinsic properties of cortical neurons (Connors et 1982; Connors, 1984; McCormick et al., 1985; Stafstron al., 1985; Schwindt et al., 1988a, 1988b, 1989; Agmon Connors, 1989; Berman et al., 1989; Chagnac-Amitai Connors, 1989; Silva et al., 1991; Spain et al., 1991a, 199 in the genesis of fast (so-called 40-Hz) and related rhyth (Llinás et al., 1991; Nuñez et al., 1992; Gutfreund et 1995; Gray and McCormick, 1996; Steriade et al., 199 Steriade, 1997). Although some investigators emphasize distinctness of some neuronal classes in displaying vari types of discharge patterns and suppose that these patte are invariant, other data show that the same neuron, eit long-axoned (corticothalamic) or short-axoned, may cha its firing elicited by depolarizing current pulses, from regul spiking to intrinsically bursting (or vice-versa) and, furth more, to fast-spiking without spike frequency adaptati

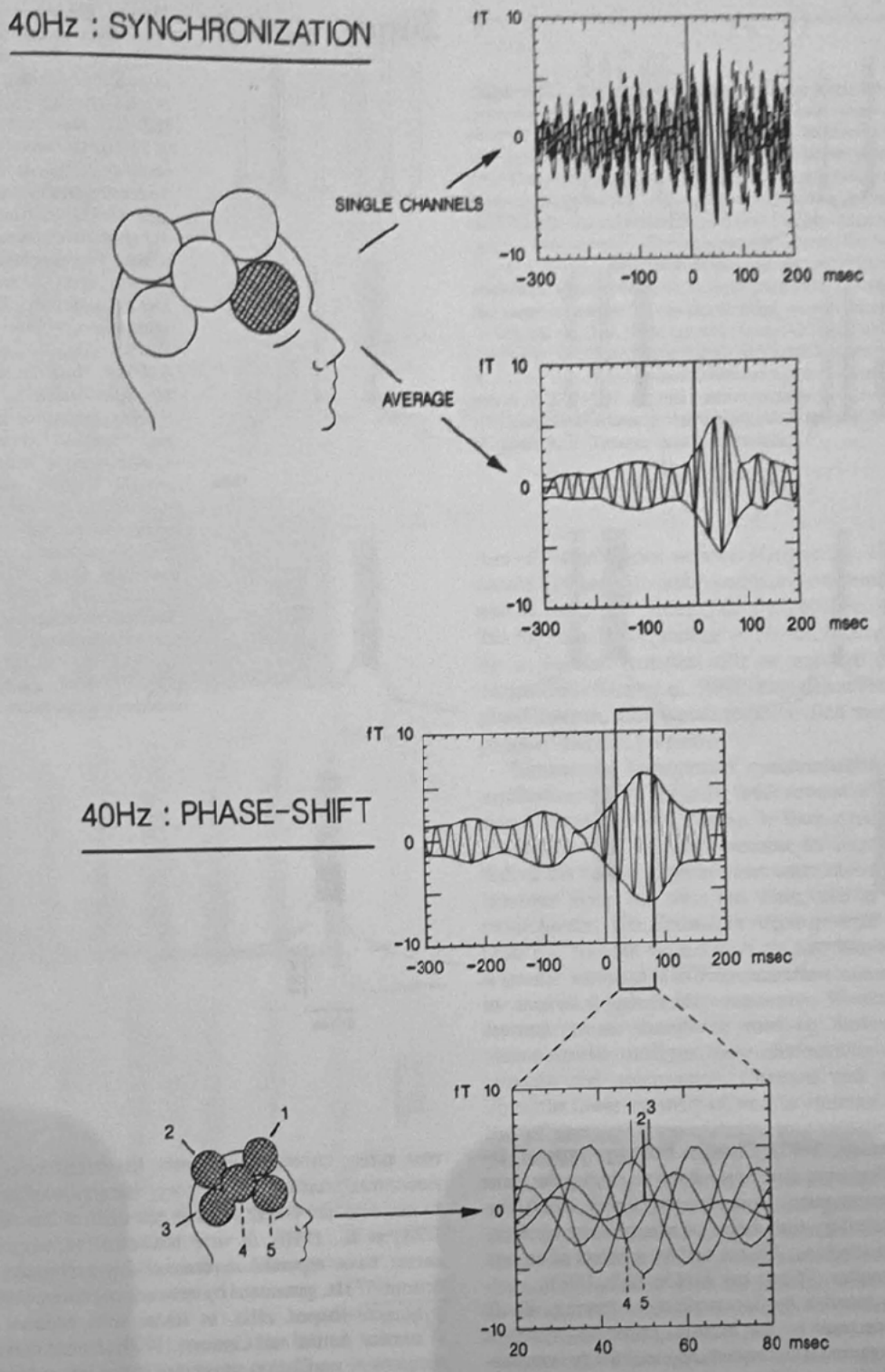


Figure 3.25. 40-Hz rhythm in magnetoencephalographic recordings from awake humans. *Top,* synchronization of 40-Hz oscillatory activity during auditory processing within seven single channels of one probe placed over lower frontal areas. The graph on the top right indicates a superimposition of 40-Hz activities, time locked to the stimulus onset, recorded from the seven channels. The graph on the lower right indicates an average of the seven individual channels, demonstrating synchronization over a large area (around 25 cm^2). *Bottom,* phase shift of 40-Hz oscillatory activity during auditory processing. The time period between 20 and 80 msec after the onset of the auditory stimuli is enlarged in the lower panel. The lowest panel shows the superimposition of averaged responses from all sensors in each of the five probe positions (hatched and numbered at left). Note the large, consistent phase shifts from region to region, indicating a continuous rostrocaudal phase shift over the hemisphere. (Modified from Llinás, R.R., and Ribary, U. 1992. Rostrocaudal scan in human brain: a global characteristic of the 40-Hz response during sensory input. In *Induced Rhythms in the Brain,* Eds., E. Basar and T. Bullock, pp. 147–154. Boston: Birkhäuser.)

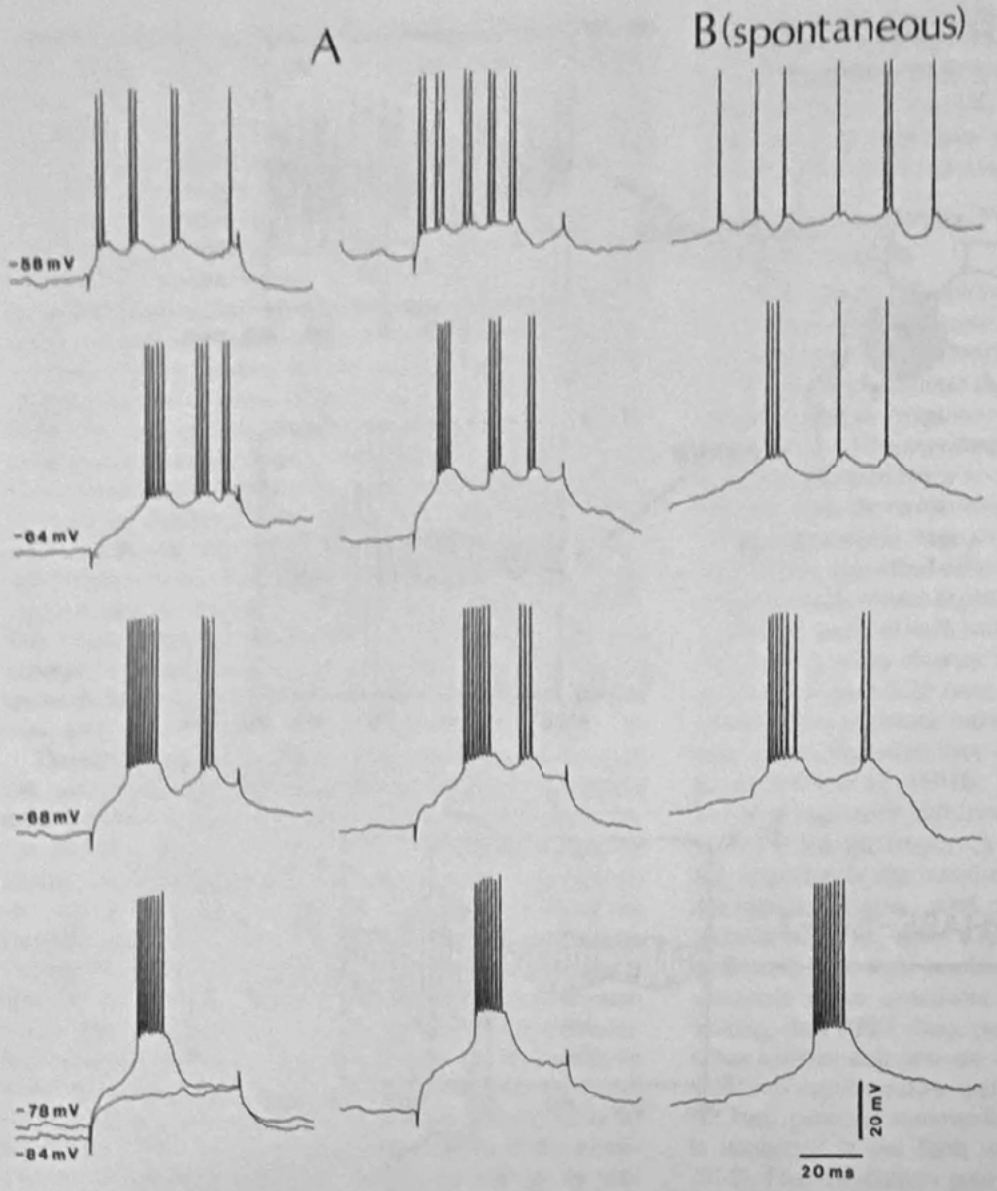
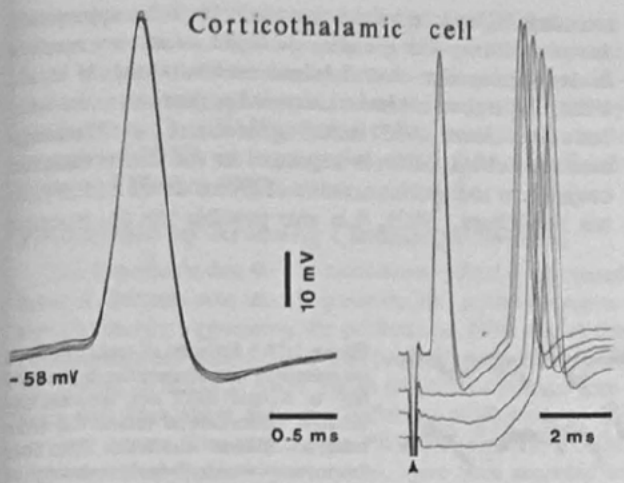


Figure 3.26. Fast oscillatory [illegible] of neuron recorded from the dorsal part of the central lateral (CL_c) intralaminar thalamic nucleus, characterized by exceedingly high-frequency (800–1000 Hz) spike-bursts recurring rhythmically at 50–60 Hz. Intracellular recording in cat under barbiturate anesthesia. A, Activities triggered by depolarizing current pulses (+1.2 nA, 50 ms) at different levels of membrane potential (V_m, indicated at left). Two examples are illustrated for each V_m level. At bottom, presence of low-threshold spike (LTS) leading to high-frequency (800–1000 Hz) spike-burst at −78 mVC and its absence at −84 mV. Note fast-recurring (50–60 Hz) spike-doublets or spike-triplets at relatively depolarized levels (−64 and −58 mV), a feature that is not seen in other types of thalamocortical neurons. **B.** Oscillatory patterns similar to those elicited by current injection occurred spontaneously at similar V_m (from top to bottom, −58, −64, −68 and −78 mV). (From Steriade, M., Curró Dossi, R., and Contreras, D. 199[illegible] Electrophysiological properties of intralaminar thalamocortical cells discharging rhythmic (~40 Hz) spike-bursts at ~1000 Hz during waking and rapid eye movement sleep. Neuroscience 56:1–[illegible]

(Fig. 3.27; Steriade, 1997). Changes from one type of discharge to another have also been observed during the same depolarizing current pulse (Connors and Gutnick, 1990), during arousal elicited by stimulation of mesopontine cholinergic nuclei (Steriade et al., 1993a), and as an effect of activating neurotransmitters (Wang and McCormick, 1993).

Despite the fact that the fast oscillations (mainly 30–40 Hz) can be generated by the intrinsic properties of single cells, complex neuronal circuits are required for the synchronization of cellular ensembles so that the fast rhythm could be expressed in multi-unit and EEG recordings. Several proposals have been made with respect to the synchronizing device of this rhythm.

The intracortical circuits were emphasized since the initial works. Freeman (1975) postulated that the generation of 40–80-Hz activity in the olfactory bulb depends on feedback inhibitory circuits involving local-circuit GABAergic neurons acting on output elements, the mitral cells. Similarly, neocortical excitatory–inhibitory circuits have been thought to underlie the 40-Hz rhythm recorded in the visual cortex (Gray et al., 1990). *In vitro* studies of rat's somatosensory cortex have reported rhythmically synchronized activities around 37 Hz, generated by networks of intrinsically bursting pyramidal-shaped cells, in slices with reduced inhibition (Chagnac-Amitai and Connors, 1989). In that study, the short duration of oscillation suggested that a limited neuronal circuit, as in cortical slices, is inadequate to sustain oscillations for long periods. Data indicating synchronization of oscillations between striate and extrastriate cortices (Engel et al., 1991b) and a callosally mediated synchronization linking neurons recorded from areas 17 of both hemispheres (Engel et al., 1991b) were the basis for the hypothesis that the mechanism generating this oscillation are exclusively located at the cortical level. In support of the idea of an interhemispheric



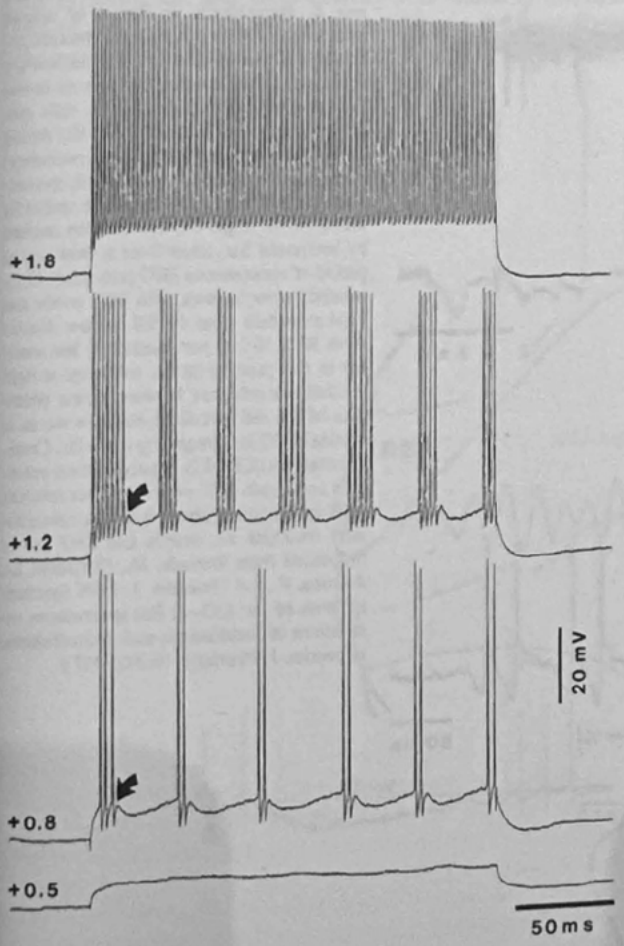


Figure 3.27. Continuum of discharge patterns elicited by depolarizing current pulses with different intensities. Intracellular recording (cat under ketamine–xylazine anesthesia) of corticothalamic neuron located in layer VI of cat suprasylvian area 7. *Top left:* Spontaneous action potentials had duration of 0.35 ms at half amplitude. *Top right:* Electrophysiological identification of thalamic projection and input: stimulus to thalamic lateral posterior nucleus (arrowhead) elicited antidromic activation (0.5 ms latency) and orthodromic spikes (2 ms latency). Five superimposed traces; the two top traces (at a V_m of −58 mV) depict both antidromic and synaptic responses, whereas antidromic spikes failed at more hyperpolarized levels. *Below*, responses of the same neuron to 200-ms depolarizing current pulses of +1.8, +1.2, +0.8, and +0.5 nA. Note fast tonic firing (420 Hz) without spike frequency adaptation (+1.8 nA); spike-bursts (intraburst frequency 400 Hz) recurring at 35 Hz (+1.2 nA); spike-doublets or triplets (interspike intervals 2.5–3 ms) at 30 Hz (+0.8 nA); and passive response (+0.5 nA). Oblique arrows (+1.2 and +0.8) point to depolarizing afterpotentials. Unpublished data by M. Steriade, I. Timofeev and N. Dürmüller.

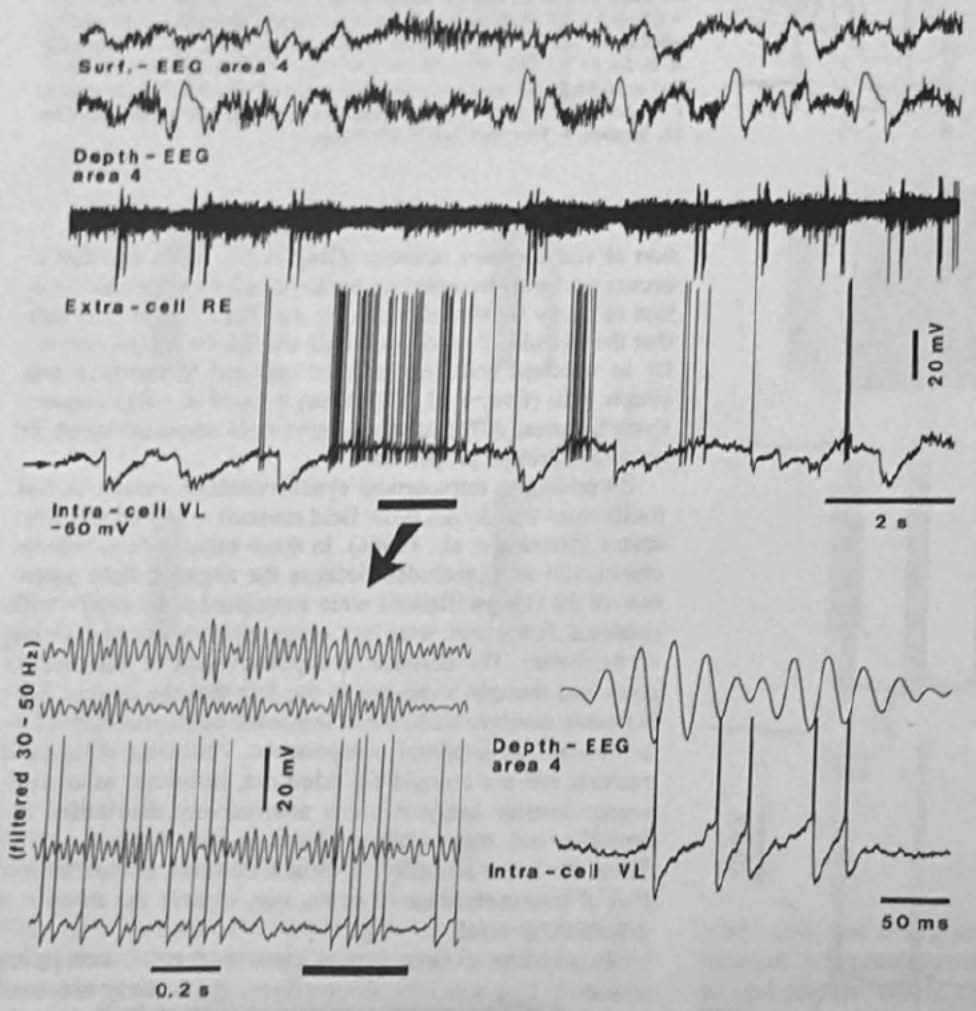
synchronization mediated by callosal projections, we were able to evoke 40-Hz oscillations upon depolarizing current pulses in cells recorded from the associational area 5 and antidromically identified as projecting to homotopic points in the contralateral cortex (Nuñez et al., 1992a). It was stressed that the 40-Hz oscillation is limited to a subpopulation of visual cortex neurons (Gray et al., 1990) and that it occurs too rarely in motor cortex to be essential for coordination of every movement (Murthy and Fetz, 1992). The fact that the stimulus-dependent 40-Hz oscillation occurs primarily in standard complex cells as opposed to complex and simple cells (Gray et al., 1990) may depend on either connectivity features, differential receptive field responsiveness, or peculiar intrinsic properties.

Surprisingly, intracortical synchronization results in fast oscillations that do not show field reversal at any depth of the cortex (Steriade et al., 1996a). In those experiments, volume conduction was precluded because the negative field potentials of the fast oscillations were associated at all depths with neuronal firing and were not observable in the underlying white matter. The absence of depth reversal of fast oscillations was thought to be due to the fact that the current flow is mainly attributable to transmembrane components and less to internal longitudinal components. Vertically distributed currents are not completely ruled out, however, as current-source-density analyses show alternatively distributed microsinks and microsources (Steriade and Amzica, 1996). Thus, the lower intensity of vertical currents, compared with that of transmembrane currents, may explain the absence of potential reversal.

In addition to intracortical circuits, there is compelling evidence that subcortical structures (particularly the thalamus) are interposed in complex neuronal chains generating the 40-Hz rhythm. The hypothesis was proposed that corticothalamic projections drive RE thalamic neurons, thus leading to 40-Hz IPSP-rebound sequences in thalamic relay cells, which reenter the cortex (Llinás, 1990). If so, during the waking state when the 40-Hz rhythm is supposed to occur preferentially (see next section), the inhibitory input from RE thalamic neurons would sculpture the tonic firing of thalamocortical cells, and rhythmic spike trains would be transmitted back to the cortex. The resetting and transiently increased amplitide of fast oscillation in TC cells by short-lasting outward pulses suggests that short IPSPs originating in GABAergic neurons may reinforce fast oscillations (Pedroarena and Llinás, 1997). The relation between RE and TC

cells in this fast rhythm is not yet elucidated, especially because, at least in felines, RE cells also have access to thalamic inhibitory interneurons. This linkage would completely transform the simple inhibitory input from RE cells to TC neurons; disinhibition may even prevail (Steriade et al., 1985).

The fact is that TC cells do oscillate at 40 Hz, due to both intrinsic and extrinsic factors. Figure 3.28 demonstrates the coherence of fast oscillations between intracellularly recorded TC cell and field potentials from the appropriate neocortical area. The presence of 40-Hz oscillatory neurons in the intralaminar central lateral nucleus (Steriade et al., 1991b, 1993c), which has widespread projections to the cerebral cortex (Jones, 1985) including the visual areas (Cunningham and LeVay, 1986), is important for the idea of thalamic conjunction and synchronization of distant cortical areas (Llinás and Ribary, 1993). It is also possible that the resonan



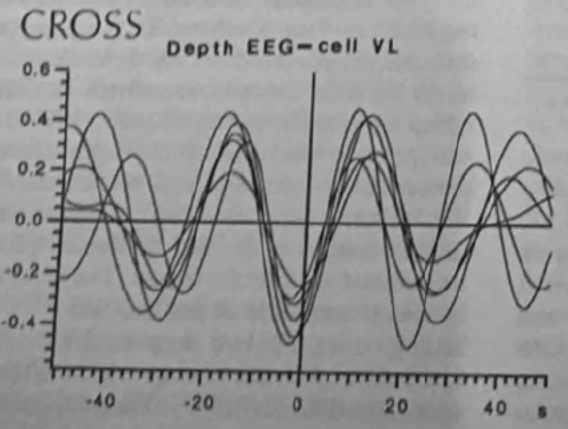


Figure 3.28. Episodes of tonic activation are associated with coherent fast rhythms (40 Hz) in cortical EEG and intracellularly recorded thalamocortical neuron. Cat under ketamine–xylazine anesthesia. *Top:* Four traces represent simultaneous recordings of surface- and depth-EEG from motor cortex area 4, extracellular discharges of neurons from the rostrolateral part of the thalamic reticular (*RE*) nucleus, and intracellular activity of thalamocortical neuron from ventral lateral (*VL*) nucleus. EEG, RE and VL cells displayed a slow oscillation (0.7–0.8 Hz) during which the sharp depth-negative (excitatory) EEG waves led to IPSPs in VL cell, presumably generated by spike-bursts in cortically driven GABAergic RE neuron. Part marked by horizontal bar, taken from a short-lasting period of spontaneous EEG activation, is expanded below (arrow), with EEG waves and field potentials from the RE nucleus filtered from 30 to 50 Hz; part marked by horizontal bar in this panel is further expanded at right to illustrate relations between action potentials of VL cell and depth-negative waves of cortical EEG at a frequency of 40 Hz. Cross-correlations (CROSS) between action potentials and depth-EEG show clear-cut relation with opposition of phase, between intracellularly recorded VL neuron and EEG wave. (Modified from Steriade, M., Contreras, D., Amzica, F., and Timofeev, I. 1996. Synchronization of fast (30–40 Hz) spontaneous oscillations in intrathalamic and corticothalamic networks. J. Neurosci. 16:392–417.)

thalamocorticothalamic circuits are only based on direct excitatory projections, requiring just one interposed synapse in layer VI. Indeed, there is a direct built-in frequency amplification in the corticothalamic pathway, as demonstrated by the fact that 30- to 50-Hz cortical volleys lead to a dramatic increase of EPSPs in target thalamocortical neurons (Lindström and Wróbel, 1990).

Potentiation by Arousing Cholinergic Systems

The hypothesis that 40-Hz oscillations reflect an increased level of alertness was tested by setting into action mesopontine cholinergic aggregates, the peribrachial (PB) area of the PPT nucleus and the LDT nucleus projecting to major thalamic nuclei. In addition, these nuclei project to the basal forebrain and, from there, they may influence the activity of the cerebral cortex. The cholinergic PB (PPT) and LDT cells, with identified thalamic projections, have been recorded in the behaving cat and their activity was found to increase during waking and REM sleep, much above the levels seen during EEG-synchronized sleep (Steriade et al., 1990a). This statistically significant increase in firing rates of PPT/LDT neurons took place 30–60 seconds in advance of the most precocious sign of EEG activation during the transition from slow-wave sleep to either arousal or REM sleep. Such a temporal correlation suggests that thalamically projecting cholinergic PPT/LDT neurons are causally involved in triggering and maintaining activation processes in thalamocortical systems.

There are two main effects of PPT or LDT stimulation upon thalamic function (Fig. 3.29). (a) Mesopontine cholinergic nuclei directly excite thalamocortical cells. This excitation has two components: a short-latency, short duration depolarization mediated by nicotinic receptors and associated with increase in membrane conductance; and a prolonged (in general 20-second, but up to 60-second) depolarization mediated by muscarinic receptors and associated with increase in input resistance (Curró Dossi et al., 1991; see also Fig. 3.23, with *in vitro* data of ACh effects by McCormick and Prince). The long-lasting muscarinic depolarization is the basis of the prolonged enhancement in synaptic responsive-

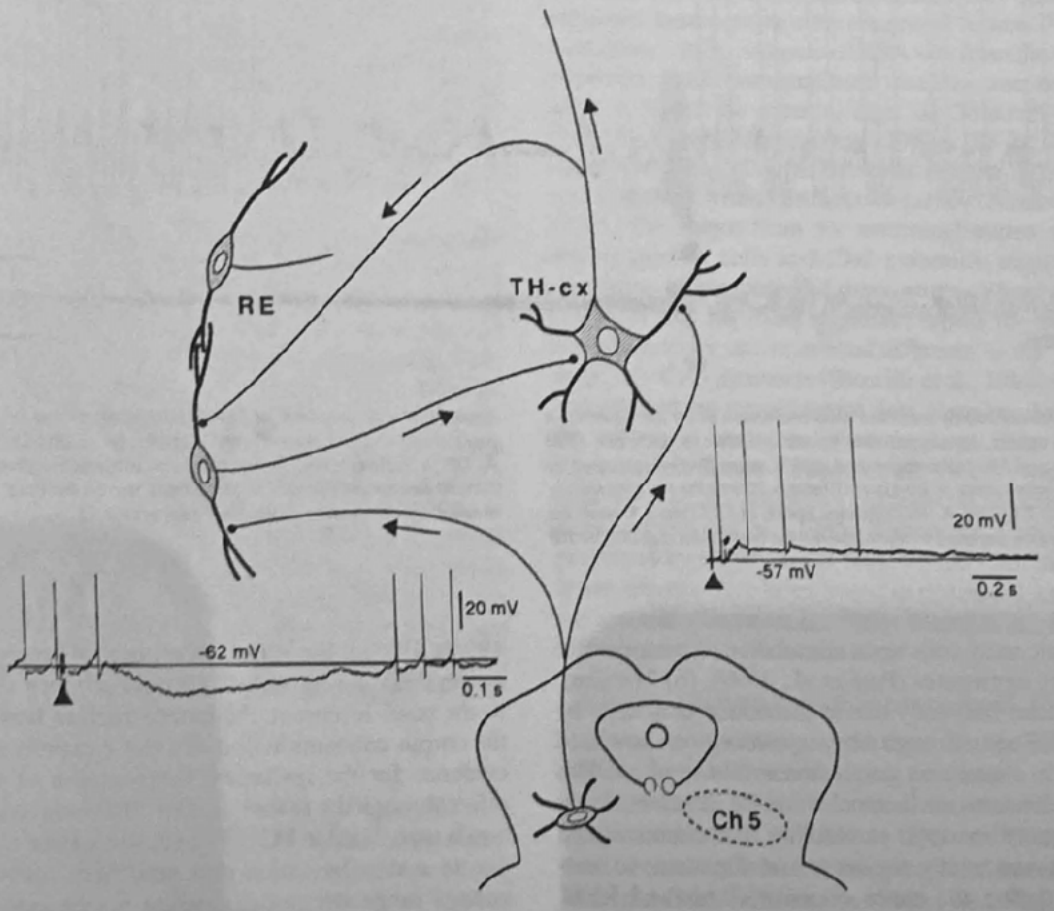


Figure 3.29. Recurrent inhibitory loop of cat thalamocortical (*TH-cx*) and reticular thalamic (*RE*) neurons, and modulation of TH-cx and RE cells by mesopontine cholinergic afferents (only the Ch5 group of pedunculopontine tegmental neurons is depicted). In insets, Ch5 stimulation (brief pulse-train, arrowhead) induces a depolarization of TH-cx neurons and a hyperpolarization of RE cells. The direct excitation of TH-cx cells is accompanied by their disinhibition (through inhibition of GABAergic RE cells). (Modified from Hu, B., Steriade, M., and Deschênes, M. 1989. The effects of brainstem peribrachial stimulation on perigeniculate neurons: the blockage of spind waves. Neuroscience 31:1–12; Paré, D., Steriade, M., Deschênes, M., a Bouhassira, D. 1990. Prolonged enhancement of anterior thalamic synap responsiveness by stimulation of a brainstem cholinergic group. J. Neuros 10:20–33; Steriade, M., Gloor, P., Llinás, R.R., Lopes da Silva, F.H., a Mesulam, M.-M. 1990b. Basic mechanisms of cerebral rhythmic activiti Electroencephalogr. Clin. Neurophysiol. 76:481–508.)

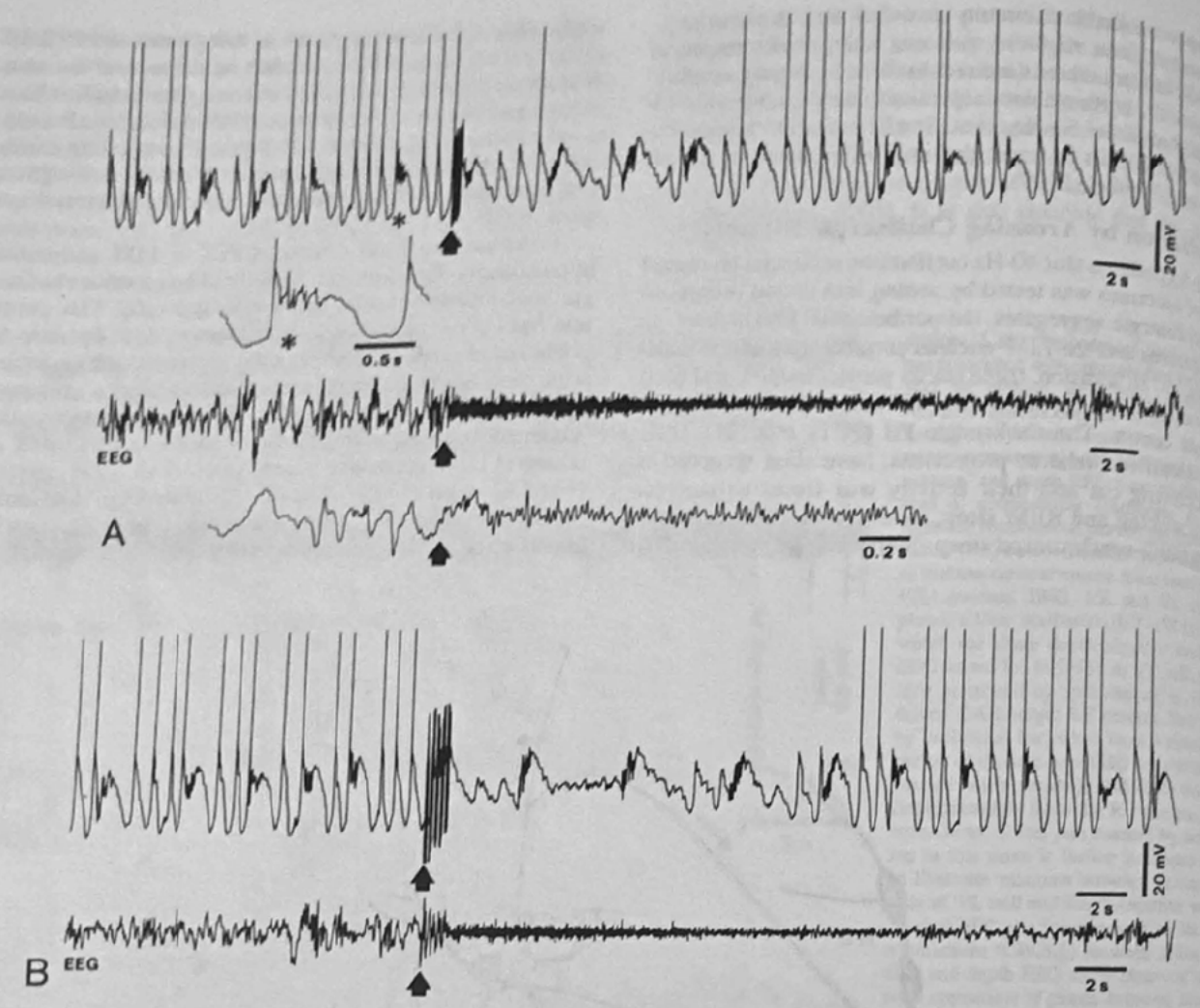


Figure 3.30. Intracellularly recorded delta oscillation in cat lateroposterior thalamocortical neuron, its suppression by mesopontine peribrachial (PB) stimulation (one and five pulse-trains in **A** and **B**, respectively, indicated by arrows), and the appearance of 40-Hz oscillations. Below the top (intracellular) trace, cortical EEG (in **A**, an expanded epoch of EEG trace around the PB pulse-train is also depicted to show the 40-Hz oscillation induced by PB stimulation). A sequence of fast depolarizing events in **A** (asterisk) is expanded and shown below. (From Steriade, M., Curró Dossi, R., and Nuñez, A. 1991a. Network modulation of a slow intrinsic oscillation of cat thalamocortical neurons implicated in sleep delta waves: cortically induced synchronization and brainstem cholinergic suppression. J. Neurosci. 11:3200–3217.)

ness of thalamic relay cells upon stimulation of mesopontine cholinergic cell aggregates (Paré et al., 1990). (b) The same cholinergic nuclei indirectly excite thalamocortical cells by inhibiting the RE cells through a hyperpolarization associated with increase in membrane conductance (Hu et al., 1989). Both these mechanisms are factors behind the increased firing rates and enhanced synaptic excitability of thalamocortical neurons, with consequently similar activated patterns in neocortical cells, during the states of wakefulness and REM sleep.

The above mechanisms of brainstem–thalamic cholinergic activation are also implicated in the potentiation of 40-Hz oscillations of TC neurons. Stimulation of the PB area in the PPT nucleus induces a twofold increase of cortical EEG waves around 40 Hz, a potentiating effect that is muscarinic-mediated as it was blocked by scopolamine (Steriade et al., 1991b, 1993a). Since mesopontine cholinergic nuclei project to the thalamus as well as through a more ventral pathway to the basal forebrain, the latter structure was destroyed and the corpus callosum was cut in those experiments to provide evidence for the ipsilateral transmission of the facilitatory effect through the thalamus. The PPT-induced 40-Hz oscillation is associated in TC cells with a blockage of slow rhythms, due to a depolarization that sets these neurons out of the voltage range where delta waves are generated (Steriade et al., 1991a) (Fig. 3.30).

These data show that, far from serving only a resonant mechanism for reciprocally coupled groups in separate columns of the sensory and motor areas, the 40-Hz rhythm reflects a condition of diffusely increased vigilance of the brain (Steriade, 1993). The role of 40-Hz rhythm in motor cortical neurons during attentive behavior was demonstrated by Mur-

thy and Fetz (1992). Fast oscillations (20–50 Hz) in cortico-thalminic networks can be conditioned in behaving animals (Amzica et al., 1997). Further cellular studies are required to show the role of this fast rhythm in thalamic and cortical sensory processes involved in selective attention.

Theta Rhythm

Theta waves have been first described in the rabbit hippocampus during arousal elicited by sensory or brainstem reticular formation stimulation (Green and Arduini, 1954). This rhythm is usually considered within the frequency range of 4–7 Hz.

The cellular bases of theta waves have been intensively investigated in rodents (see below) but this rhythm is less evident in other mammals. In cat, the theta hippocampal activity is quite conspicuous during REM sleep, but occurs only exceptionally during wakefulness (Jouvet, 1965). It was also claimed that theta rhythm decreases in amplitude and regularity from rodents to other species and is poorly represented in primates (Crowne and Radcliffe, 1975; but see Stewart and Fox, 1991). As to humans, the presence of this rhythm was denied, even with deep electrodes inserted in the hippocampus (Brazier, 1968; Halgren et al., 1978, 1985). The normal theta activity, generally considered as poor or absent in primates, should not be confused with the so-called "pathological theta waves," described as a slowing down of alpha activity, due to great reduction in cerebral blood flow (Ingvar et al., 1976), to metabolic encephalopathies (Saunders and Westmoreland, 1979), or occurring after disturbances in deep midline structures (Gloor, 1976).

Because of its preponderance in rodents, this section will only briefly summarize the origins and cellular mechanisms of theta waves in these species. The importance of theta rhythm transcends its relations with sensory processing and the control of different types of movements in rodents (Grastyan et al., 1959; Vanderwolf and Leung, 1983; Buzsáki, 1996) (Fig. 3.31). Indeed, the induction of long-term synaptic potentiation (LTP), a phenomenon involved in the beginnings of memory (Lynch, 1987), is optimal when the time interval between stimuli is about 0.2 second, which corresponds to the frequency band of hippocampal theta (Larson et al., 1986; Larson and Lynch, 1986; Pavlides et al., 1988). It was hypothesized (Larson and Lynch, 1986) that, at this frequency, the facilitation occurs because there is a suppression of IPSPs and a prolongation of the depolarization, resulting in an increased influx of calcium ions that would lead to an amplification of LTP.

Origins

Theta waves have been found in cortical limbic areas (such as hippocampus, entorhinal cortex, and cingular areas) where they are generally described as rhythmic slow activity (RSA) extending from 3–4 Hz to 10 Hz. The term *RSA* is used to avoid the term *theta rhythm* which indicates a less broad frequency spectrum (4–7 Hz).

Some experimental results have suggested that the master structure controlling at least one type of theta activity is the septohippocampal cholinergic system, driven from the brainstem reticular core (Petsche et al., 1965). The rostral course of the ascending brainstem system triggering synchronized theta waves was traced by Vertes (1971, 1982) and the presence of theta-on cells in the caudal diencephalon was reported by Bland et al. (1995). Cellular recordings in the ventral part of the medial septum and the vertical branch of the diagonal band nucleus have shown that about half of these elements discharge in close time-relation with hippocampal RSA (Apostol and Creutzfeldt, 1974; Assaf and Miller, 1978; Gatzelu and Buño, 1982; Lamour et al., 1984). However, septal bursting cells are phase-locked with the hippocampal RSA even after fimbria cuts that interrupt the connections between septum and hippocampus (McLennan and Miller, 1976). On the other hand, lesions of the medial septum lead to the disappearance of hippocampal RSA (Petsche et al., 1962; Vinogradova et al., 1980). These data were at the basis of the claim that septum is the pacemaker of theta rhythm. Nonetheless, the local hippocampal circuits actively contribute to the genesis of theta waves as the application of the cholinergic agonist carbachol in hippocampal slices produces waves within the frequency range of theta (Konopacki et al., 1987).

Since atropine does not completely suppress theta activity, an additional (noncholinergic) system is believed to arise in the entorhinal cortex. Indeed, atropine totally eliminates hippocampal theta activity after entorhinal lesions (Vanderwolf and Leung, 1983). A dipole of RSA was found in the entorhinal cortex, with two amplitude maxima, one superficial in layers I–II and the other in layer III (Mitchell and Ranck, 1980; Alonso and Garcia-Ausst, 1987a, 1987b; Boejinga and Lopes da Silva, 1988). Stellate cells in layer II display intrinsic oscillations within the theta frequency (Alonso and Llinás, 1989). The output from the entorhinal cortex activates the dentate granule cells and CA1 pyramids, regarded as main generators of hippocampal theta waves (Bland et al., 1980). The view that the most important inputs for the generation of theta activity are entorhinal afferents to the granule cells and CA1–CA3 pyramids (Buzsáki et al., 1983; Leung, 1984) is supported by experimental data showing that removal of the entorhinal input abolishes the large theta dipole in the hippocampal fissure (Ylinen et al., 1995). Another theta dipole is set up by inhibitory currents on pyramidal-cells' somata (Buzsáki et al., 1986; Soltesz and Deschênes, 1993) generated by hippocampal local-circuit inhibitory cells. Different dipoles have been found in restrained and freely moving animals (Green et al., 1960; Bland et al., 1975; Winson, 1974).

Cellular Mechanisms of Hippocampal Theta Waves

The discrepancies prevail over the agreements as to the cellular mechanisms underlying the genesis of the hippocampal theta waves. The first intracellular study showed that 85% of pyramidal cells impaled in CA1 and CA2 fields display oscillations of the membrane potentials that are synchronous with the field theta waves (Fujita and Sato, 1964). These authors considered the theta rhythm as mainly due to rhythmic EPSPs. The somatic hyperpolarizations were believed to result from the recurrent collateral activation of local-circuit inhibitory cells following the rhythmic discharges of pyramidal neurons driven by septal afferents. In addition to this recurrent inhibitory circuit, interneurons in the CA1 area are probably directly excited by septal inputs

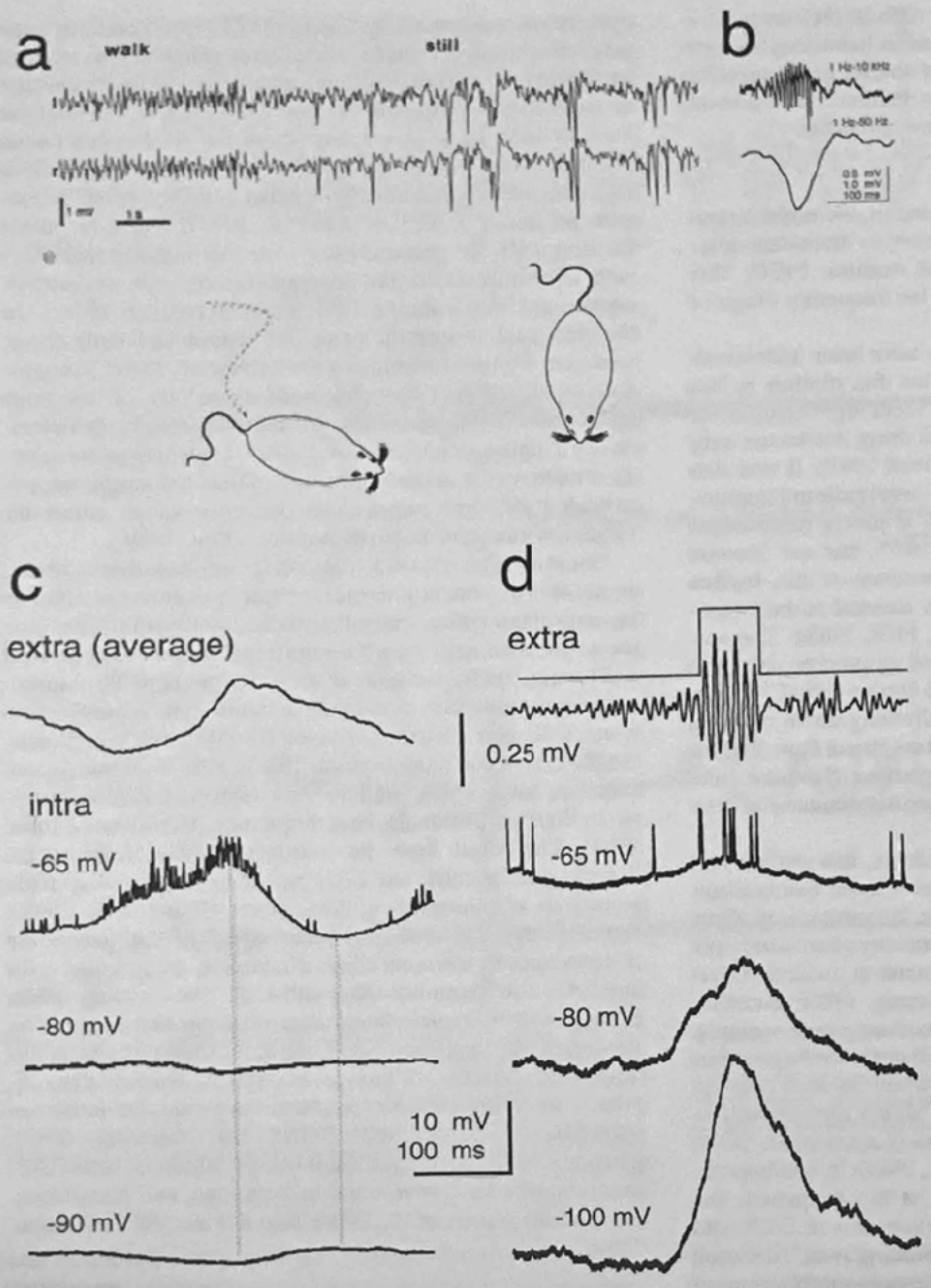


Figure 3.31. Behavior-dependent ... scopic (EEG) states in the hippocampus ... their intracellular correlates in the rat. (**a**) ... tracellular recordings from the CA1 ... radiatum of the left (l) and right (r) hippo... pus during the transition from explor... (walk) to being still (still). Note regular ... waves during waking and large, negative ... waves (SPW) during immobility. Note also ... SPWs are bilaterally synchronous. (**b**) A ... SPW with simultaneously recorded fast ... oscillation from the CA1 pyramidal layer ... faster time scale. (**c**) and (**d**) Intracellular ... lates of field theta (**c**) and SPW (**d**) in ... pyramidal cells under urethane anesthesia. ... (**c**) extracellular and intracellular averages ... theta (n = 80 to 200 repetitions) at three ... ent polarization levels. Note that hyperpolariza... tion leads to a reversal of intracellular ... (dotted lines) at chloride equilibrium potential ... (details not shown). In (**d**) a single ripple ... (extra) showing the trigger pulse used for ... cellular (intra) averaging of SPW events. ... that intracellular hyperpolarization reveals ... strong depolarization force during the ripple. ... Intracellular traces are averages of 10 to 20 ... etitions. (Modified from Buzsáki, G. 1996. ... hippocampal–neocortical dialogue. ... Cortex 6:81–92.)

as well as by entorhinal inputs traveling in the perforant path (Buzsáki et al., 1983).

Whereas the prevalent view is that IPSPs are major events in CA1 pyramidal cells during RSA (Leung and Yim, 1986; Soltesz and Deschênes, 1993; see also Fig. 3.31c), Nuñez et al. (1987) claimed that RSA reflects EPSPs and presumably calcium-mediated slow spikes in CA1 and CA3 pyramids (Fig. 3.32). The majority of CA1 pyramidal cells undergo a sustained depolarization associated with a decrease in membrane conductance. Those authors concluded that the IPSPs do not play an essential role in the genesis of the focal theta waves. Thus, at the present time, there is no full agreement as to the PSPs and intrinsic currents involved in the different wave patterns of hippocampal RSA. Future studies should attempt simultaneous recordings from inhibitory interneurons and pyramidal cells to ascertain whether IPSPs contribute to the field theta activity. It was proposed that the sustained depolarization of pyramidal neurons during theta is due to a disinhibitory process caused by a septum-induced suppression of tonic inhibition arising in hippocampal local-circuit inhibitory interneurons (Krnjevic and Ropert, 1982; Krnjevic et al., 1988).

Because the main origin of EEG waves should be searched in the synchronized PSPs and simultaneous intracellular re-

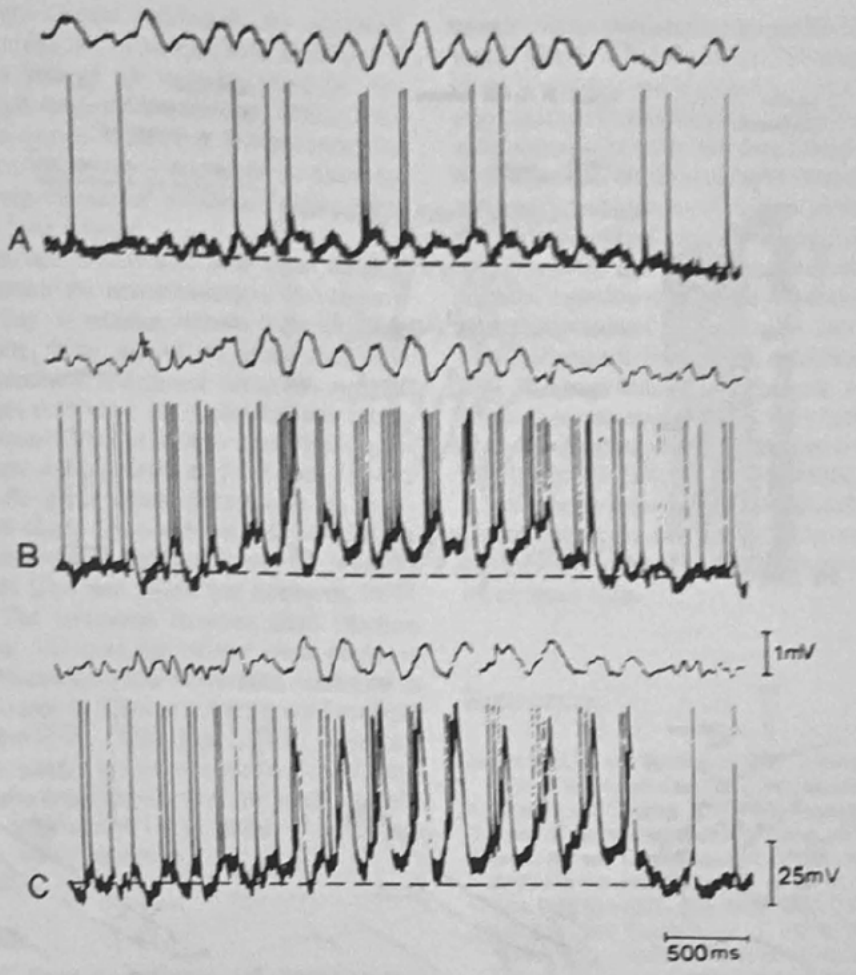


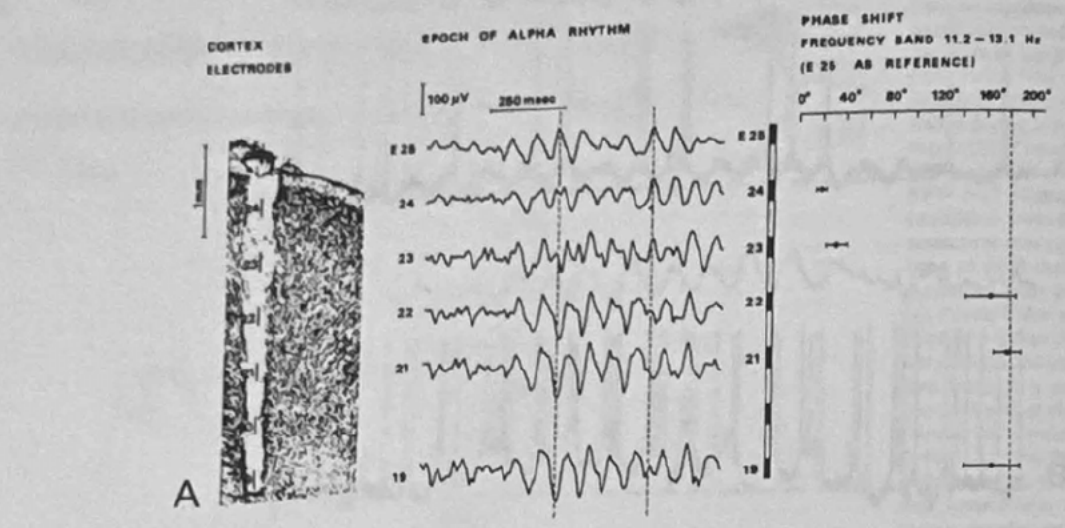
Figure 3.32. Theta rhythm generation. Hippocampal EEG (upper trace) and intracellular records of hippocampal pyramids (lower trace) in rats under urethane anesthesia. **A** and **B**. Two different CA1 pyramids; **C**, from CA3 pyramid. With theta waves, sustained depolarization above "resting" level (broken lines) is present in all cases. Smooth sine-like theta waves were recorded without (**A**), with occasional (**B**), or with continuously rhythmic (**C**) slow spikes. (Modified from Nuñez, A., Garcia-Austt, E., and Buño, W. Jr. 1987. Intracellular theta rhythm generation in identified hippocampal pyramids. Brain Res. 416:289–300.)

cordings from a great number of neurons are impossible at this time, computer simulations have been used to produce oscillations similar to those observed in living animals. Traub and his colleagues (Traub et al., 1987; Miles et al., 1988; Traub et al., 1989; Pedley and Traub, 1990) have used models of the CA3 area with 200–20,000 pyramidal neurons as well as bursting and nonbursting inhibitory cells. Besides, they have endowed pyramidal neurons with slow inward currents to produce spontaneous spike bursts. Such a system displays a highly organized rhythmic activity at the population level, while individual elements show chaotic firing. These synchronized activities are similar to those seen experimentally.

Alpha Rhythm

This chapter ends with an account of what is presently known about the origins of alpha rhythm occurring in the frequency range of 8–13 Hz. Although this probably is one of the few most important grapho-elements and its description dates back to Berger (1929), there is no knowledge about its cellular mechanisms because alpha waves have been mainly analyzed from scalp recordings in humans and laminar profiles of cortical field potentials in animals. For a description of different EEG patterns of alpha rhythms in the waking adult, see Niedermeyer (1987).

The temptation to understand the mechanisms of this rhythm at the cellular level by recording spindle oscillations under barbiturate anesthesia (Andersen and Andersson, 1968) is understandable, but alpha and spindle waves are quite different oscillations. Indeed, while the frequencies of these two rhythms may overlap, their origins and especially their behavioral context are dissimilar. Alpha waves are usually described as occurring during relaxed wakefulness. Some studies refer to them as a central timing mechanism regulating afferent and efferent signals (Sanford, 1971). The conventional wisdom that the occipital alpha rhythm is associated with reduced visual attention is challenged by the fact that the incidence of alpha waves increases during responses to visual stimuli or concentration on visual imagery (Mulholland, 1969) and the reports about augmentation of alpha activity during attention tasks (Creutzfeldt et al., 1969; Ray and



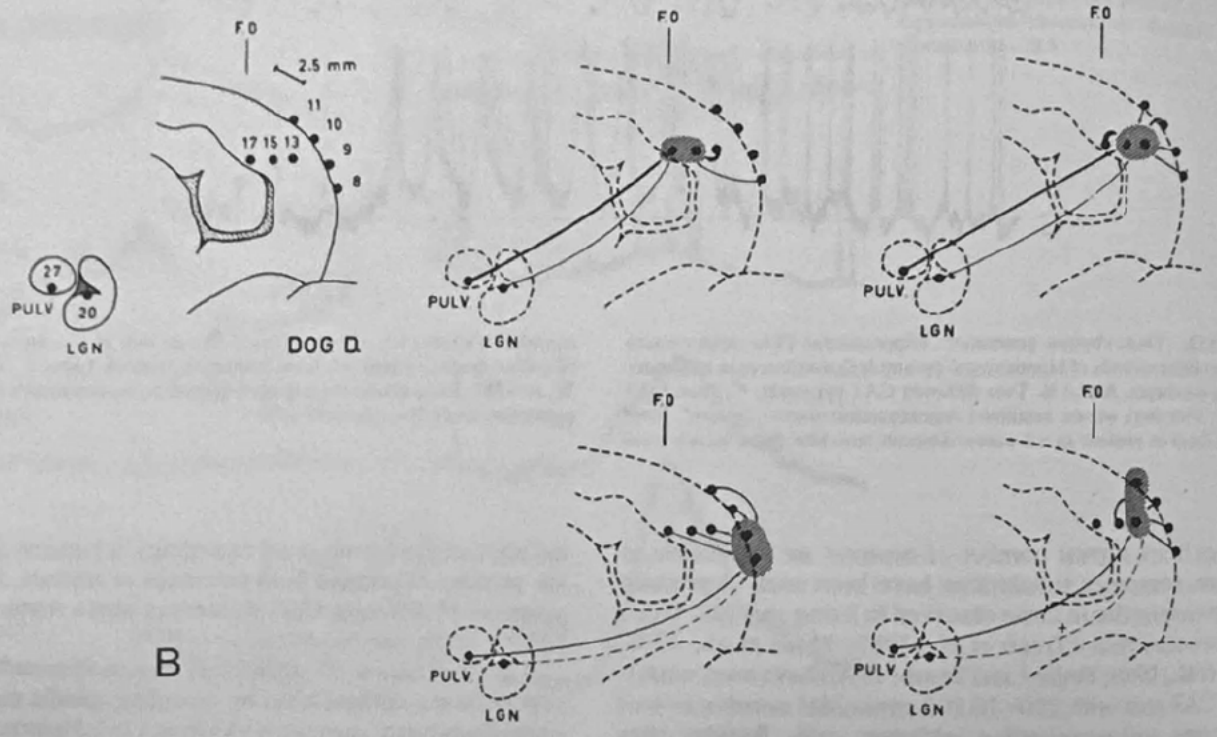


Figure 3.33. Alpha rhythm in dog. **A.** *Left:* Photomicrograph of a section of the marginal gyrus (visual cortex). The electrode bundle consisted of 7 wires with a bare tip of 0.15 mm. *Middle:* An epoch of alpha activity recorded simultaneously from 6 sites corresponding to those at left, against a common frontal cranial reference (negativity upwards). Note that there is a polarity change between the most superficial sites (E25, 24) and the deepest sites (E22, 21 19). *Right:* Phase shift between the most superficial site (E25) and deeper lying sites computed by using spectral analysis based on the average of a number of epochs within the frequency band 11.2–13.1 Hz. The mean values of the phase shift and the corresponding standard deviations are indicated. **B.** Schematic view of the relationship between thalamic and cortical alpha activity. The lines indicate the amount of influence that a thalamic signal has on the coherence between a pair of cortical signals (shaded recorded during alpha activity. This was measured by partializing the cortical coherence on the thalamic signals from the lateral geniculate n (*LGN*) and pulvinar (*PULV*) or other cortical signals. Despite the fa PULV has an influence on cortico-cortical domains of alpha activit intercortical factors play a significant role for the establishment of co domains of alpha activity. (Modified from Lopes da Silva, F.H., and S van Leeuwen, W. 1977. The cortical source of alpha activity. Neu Lett. 6:237–241; Lopes da Silva, F.H., Vos, J.E., Mooibroeck, J., and Rotterdam, A. 1980. Relative contribution of intracortical and thalamo cal processes in the generation of alpha rhythms, revealed by partial c ence analysis. Electroencephalogr. Clin Neurophysiol. 50:449–456.)

Cole, 1985). Distinctly, spindle oscillations are associated with unconsciousness and are associated with blockage of synaptic transmission through the thalamus from the very onset of sleep (Steriade et al., 1969; Steriade, 1991). Thus, the idea of an alpha-to-spindle continuum, or alpha viewed as an embryo of spindle oscillations, is untenable. As discussed below, not only the significance of spindle and alpha waves is different, but also their origins.

Lopes da Silva and his collaborators provided a series of experimental data toward the understanding of the origin of alpha rhythm in the dog. At variance with the thalamic origin of spindle oscillations, these authors suggested that alpha waves are mainly generated and spread within the cerebral cortex. Their data are as follows. (a) Alpha rhythms can be recorded from the visual cortex as well as from visual thalamic (lateral geniculate and pulvinar) nuclei (Lopes da Silva et al., 1973). (b) In the visual cortex, alpha waves are generated by an equivalent dipole layer centered at the level of the somata and basal dendrites of pyramidal neurons in layers IV and V (Lopes da Silva and Storm van Leeuwen, 1977) (Fig. 3.33A). (c) The coherence between alpha rhythms recorded in adjacent (~2 mm) foci of the visual cortex is larger than in any thalamocortical coherences measured in the same animal (Lopes da Silva and Storm van Leeuwen, 1978; Lopes da Silva et al., 1980) (Fig. 3.33B). These data led to the conclusion that a system of surface-parallel intracortical connections is mainly involved in the spread of alpha activity, while the influence of visual thalamic nuclei over the cerebral cortex is only moderate.

Final Remarks

The tendency of some investigators and practitioners to use the terms of various EEG rhythms by referring only to their frequency bands is misleading. Some points should be made: (a) EEG rhythms must be understood in the context of interconnected brain networks allowing synchronized activities of neuronal ensembles; (b) each rhythm should be placed within the context of distinct behavioral state; and (c) the knowledge of cellular mechanisms underlying various types of EEG oscillations is compelling. The last point is illustrated, for example, by the fact that various (slower and faster) types of spindle oscillations are easily understood on the basis of different durations of hyperpolarizations in TC cells, without calling for qualitatively different rhythmic activities.

The electrophysiologists interested in brain rhythmic activities perform analytical researches of central oscillations by using intracellular recordings in *in vivo* preparations under various anesthetics or *in vitro* slices. Intracellular recordings of thalamic and cortical cells in unanesthetized preparations are rarely used in relation with different behavioral conditions; the difficulty to obtain stable recordings and to monitor the membrane potential in an animal with intense synaptic bombardment cannot be overemphasized. The *in vivo* studies on anesthetized animals and the *in vitro* works have provided the most reliable data on neuronal bases of EEG rhythms. This chapter is mainly built on their intracellular results. However, some anesthetics do not faithfully mimic the complex state of the EEG-synchronized sleep; for example, barbiturates induce overwhelming spindle oscillations that mask slower (0.1 to 4 Hz) rhythms. The ketamine–xylazine anesthesia is probably the best tool to obtain slow oscillations, in association with spindle waves, as well as fast rhythms during epochs associated with cells depolarization. The *in vitro* techniques provide the most reliable data on intrinsic properties and ionic conductances of various cellular types. Of course, the very simplified circuitry in thin slices is far from the situation in the real nervous system, where a given structure displays a peculiar rhythm due to intrinsic membrane conductances and network properties, but also undergoes influences from a connected brain region exhibiting a different rhythmicity. Although brain slices represent a hypersimplified condition, some investigators use such terms as *spindles*, *alpha*, or *slow waves*, only because of remote frequency similarities and despite the fact that the described rhythms have little to do with the patterns of bioelectrical activity in living animals. Future techniques will undoubtedly combine the opportunities provided by *in vitro* slices with the preserved connectivity of an intact brain.

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EXHIBIT 5

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COVER EDITORIAL

The inventor of electroencephalography (EEG): Hans Berger (1873–1941)

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Introduction

German psychiatrist Hans Berger (Fig. 1) was born on 21 May 1873 in the town of Neuses near Coburg, in southern Germany [1, 2]. To become an astronomer in 1892, he enrolled at the Friedrich Schiller University of Jena as a mathematics student. He left after one semester and served in the army for 1 year as a cavalry. He was injured by falling from his horse during a training. Meanwhile, his sister, who was miles away from Berger, felt that he was in bad condition and she sent an urgent telegram. Berger then considered this is a telepathic communication. Therefore, Berger had a desire to study psychiatry [2, 3]. After receiving the degree of Doctor of Medicine at the University of Jena in 1897, he began working as an assistant to Otto Ludwig Binswanger (1852–1929), who served on the department of psychiatry and neurology at the Jena clinic [3]. Berger became the head of this clinic in 1919 [1].

When Berger, who was very shy and had poor social relations, returned to Germany, he encountered various difficulties. He committed suicide on 1 June 1941 as a result of severe depression [1, 3].

The discovery of EEG

Berger's incident led him to explore the physiological basis of psychic events. However, the results were not as expected. He began researching the brain's electrical activity. Berger's son Klaus and daughter Ilse became subject in all his work [1]. Berger previously recorded brainwaves by injecting Novocaine into the scalp and placing the electrode in the periosteum [3]. He recorded his first EEG on July 6, 1924, on a 17-year-old boy during a neurosurgery performed by neurosurgeon Nikolai Guleke (1878–1958) [1, 4]. In later periods, he developed a recording technique, connecting the electrode to the scalp as it is now done, and he was the first person to record as non-invasive the electrical activity of the human brain [1]. In 1929, he published his first paper on EEG, "Über das Elektrenkephalogramm des Menschen," using the terms alpha and beta waves (the alpha waves also known as Berger wave) [3, 5]. In this article, Berger examined the EEG recordings of patients who was different ages and genders (Fig. 2 and Cover) [5, 6]. With his studies, Berger recorded that brainwaves change during a mental activity and during sleep. He also observed that there were different electrical waves around the tumor [3].

Conclusion

Today, EEG is known as an important method routinely used in diagnosis of diseases in many fields such as neurology and psychiatry. Hans Berger should be commended for this important discovery.

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EX. 5
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Fig. 1 Hans Berger which can be found at https://en.wikipedia.org/wiki/Hans_Berger[05.02.2020]

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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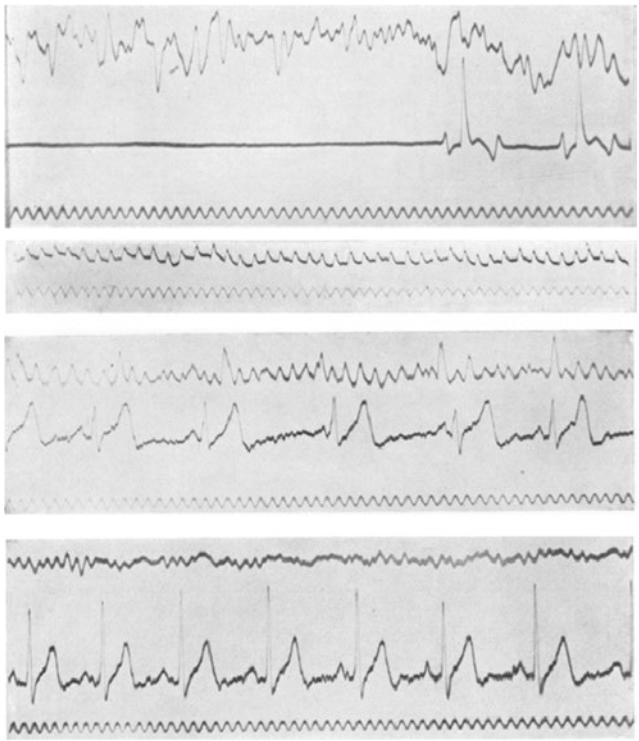

Fig. 2 and Cover. Examples of EEG that Hans Berger recorded from patients of different genders and ages in his paper *Über das elektroenkephalogramm des menschen*

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EXHIBIT 6

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THE INTERPRETATION OF POTENTIAL WAVES IN THE CORTEX.

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(*Received April* 4, 1934.)

If two electrodes are placed on the surface of the brain a record of the potential difference between them shows repeated fluctuations which may be as large as a millivolt. Recent work on this subject dates from the string galvanometer investigations of Prawdicz-Neminsky [1913, 1925], and in the last five years a number of papers have been published from various laboratories. A review of the literature prior to 1932 has been given by Fischer [1932]. It has been shown that the potential waves are generated in the cortex, that they are modified by stimulation of sense organs and abolished by cutting off the oxygen supply to the brain; in fact there is no doubt that they are due to the activity of the cortical neurones. Recording them would seem to offer the most direct method of investigating cortical activity, but for the difficulty that they are certainly summated effects compounded out of the potential changes in many neurones. It is most unlikely that the change in each neurone is an exact copy, on a smaller scale, of the massed effect, and we cannot go much further until we know how the massed effect is built up.

The problem, then, is to decide what kind of changes to expect in the individual neurones. The potential waves recorded in the cortex are usually irregular and show wide variations in size and duration. Are the action potentials of the cortical cells as variable, or are they as uniform as the impulses in a nerve fibre? If the potential between two points on the brain increases for half a second and then decreases, are we to suppose that potential gradients have developed equally gradually in certain neurones, or that the effect is due to repeated brief outbursts which increase in number as more and more units are involved and as their activity rises? The question has been raised before by one of us [Adrian, 1931] in connection with the slow potential changes in the nerve ganglia

of insects. It was suggested that the depolarization of a nerve cell might be a much more gradual process than the explosive change which occurs in the nerve fibre. In the present work, however, we have found no evidence in favour of very slow changes in the individual neurones, and we have reached the conclusion that the cortical effect is mainly built up out of repeated brief pulsations, slower than the potential waves in a nerve fibre but of the same general character.

TECHNIQUE.

All our experiments have been made on animals under a general anæsthetic. The bearing of this on the results will be discussed later; for the present it is enough to say that the anæsthetized brain shows considerable electrical activity and that other workers have found no evidence of a radical change in the character of this activity when consciousness is lost. A few experiments were made on cats; the majority on rabbits under chloroform and ether, or urethane. Chloralose, dial or pernocton were used occasionally.

The brain was exposed by trephining the skull over the parietal region and enlarging the opening to about 2×2 cm. Bleeding from the bone was controlled by plasticine, the dura was opened and turned back, and the surface of the brain was repeatedly irrigated with warm Ringer. The head was held in a clamp but the body was not secured in any way. In some experiments respiration was recorded by a tambour connected to a branch from a tracheal cannula and the electrocardiogram by leads on the head and in the anus.

For leading from the surface of the cortex the electrodes were usually of the non-polarizable type in which a coil of silver wire coated with $AgCl_2$ dips into a glass tube containing Ringer's fluid. For many experiments we used tubes plugged at the lower end with china clay through which a worsted thread protruded to make contact with the cortex. For leading from very small areas with electrodes close together the tubes were drawn out to a point with an external diameter of 1 mm. or less and plugged with gelatin, the orifice of the tube resting lightly on the cortex.

The concentric needle system [Adrian and Bronk, 1929] gives the most restricted interpolar field, but we have found it troublesome to use on the brain. If the outer tube and inner wire are both of silver the area which can be coated with $AgCl_2$ is not large enough; the electrodes often develop a high resistance after they have been in contact with the brain for 10–20 min. If the tube and wire are not of the same metal, local circuits are often set up and may produce troublesome artefacts. Pointed gelatin electrodes are more reliable, though we have not tried to develop a concentric type.

For leading from the deeper layers of the cortex we have used both the pointed gelatin type and the concentric needle system (of silver), but many records have been made with a large indifferent electrode on the surface and an enamelled silver wire, bared at the tip and coated with $AgCl_2$. This could be thrust through the cortex with a minimum of damage.

The methods we have used for analysing the cortical waves have depended mainly on the comparison of simultaneous records from three

pairs of electrodes with three independent amplifying systems and three oscillographs. To begin with the amplifiers were of the usual type, one of the input leads being connected with the grid of the first valve and the other to earth. Thus one of each pair of leads was earthed, and as nothing was to be gained by using three distinct earthed electrodes we used a common one of large area on the cortex or in the mouth, with separate electrodes to the three grids. It was soon obvious that such an arrangement gave unsatisfactory results. The brain is a large mass of tissue in which all manner of potential changes are taking place; when the grid electrode rests on a point on its surface and the other is earthed and forms a diffuse lead from the whole mass, there is no reason to suppose that a change in their relative potentials is due to changes occurring at the point where the grid electrode is applied. The brain at this point may be inactive and the average potential of the rest of the brain (relative to the point) may have altered. With three pairs of electrodes, one of each pair going to a common earth, or with three grid electrodes and a single diffuse earthed lead the position is much the same—the simultaneous appearance of a potential wave at the three pairs does not necessarily imply simultaneous activity at the three regions to which they are applied. For this reason it is exceedingly difficult to draw any certain conclusions from the records. The difficulties which arise in this way are illustrated by a control experiment made on the frog with three concentric needle electrodes. The outer tube of each needle was earthed, the inner wires leading to the grids of the three amplifiers. With this arrangement if one needle system was on the heart and the other two on the liver, all three showed potential waves corresponding to the electrocardiogram. A single needle system on the liver showed only a minute disturbance, but connecting the outer tube with electrodes in other parts of the body caused its potential to fluctuate relative to that of the inner wire and so brought back the wave at each heart beat.

To overcome the difficulties introduced by the common earthed lead we adopted an amplifying system in which neither input lead was earthed. The system [Matthews, 1934] has been described elsewhere in detail, as it has some additional advantages over the usual form. Two input valves are used in a balanced circuit, the two electrodes lead to the two grids and the resulting changes in anode current are handed on through condensers to the later stages of the amplifier, which are of the usual condenser coupled type. With this arrangement the three pairs of electrodes are quite independent; a pair on the frog's liver shows only a very small deflection, although other pairs on the heart give simultaneous records

of the electrocardiogram (Fig. 1). Three Matthews' oscillographs were used to record the potential changes, which were photographed and could be viewed with the usual rotating mirror arrangement. A loud-speaker was not often used, as the cortical potentials often rise and subside too slowly to give clearly audible sounds. Our records of the cortical potentials are sometimes slightly distorted by the use of capacity-coupled amplifiers, but this has been controlled by the use of a battery-coupled amplifier (see p. 455) and the distortions are not of a kind which would affect the interpretation of the records.

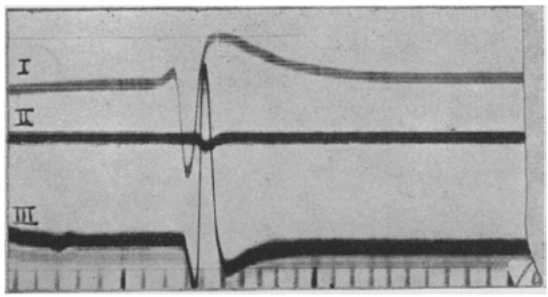

Fig. 1. Frog's electrocardiogram with balanced amplifiers. Three concentric needle electrodes, I and III on the heart, II on the liver. Sensitivity the same for all. Time marker gives 1/20 sec. With three earthed amplifiers the deflections from the liver are as large as those from the heart.

Results.

Characteristic potential changes from the cortex.

(*a*) *Rhythmic outbursts in deep anæsthesia.* In an animal which is lightly anæsthetized and still more in one which is not under a general anæsthetic [*vide* Fischer, 1932, 1933; Kornmüller, 1933; Tönnies, 1933] the potential changes in the cortex are irregular and may be difficult to predict. Under deep anæsthesia they become much more regular, and although there are variations from one rabbit to another we can make fairly certain of recording the same type of disturbance again and again over periods of several hours. We had not expected this result, though it is mentioned by Bartley and Bishop [1933]. It has been of great value, for it has enabled us to plan experiments knowing what kind of cortical response to expect.

Fig. 2 illustrates, in A, the type of record usually found in light urethane anæsthesia, and in B and C, the more regular type with deeper anæsthesia. In the latter a series of outbursts occur at intervals of 1–3 sec. Each consists of one or more large elevations rising and declining relatively slowly with a series of much smaller and briefer oscillations superimposed on them. The slow elevations represent potential changes of 0·5–1·0 millivolt. If the amplification is of the order needed to keep the whole of these waves on the record there is usually no sign of activity in the intervals between each outburst, but with higher amplification it is possible to detect oscillations of the brief type scattered through most of the quiet interval.

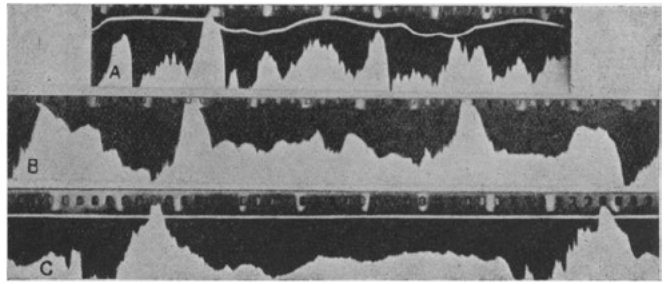

Fig. 2. Potential changes from cortex of rabbits under urethane anæsthesia. A, moderately light; B and C, deep. In deep anæsthesia the slow waves become more regular. The "brief" waves are just visible. Condenser-coupled amplifier, non-polarizable thread electrodes 1 cm. apart on cortex. White line in record A signals respiration. Time marker gives 1/4 sec. intervals.

Under urethane the outbursts seldom occur with complete regularity; more definite rhythms appear in rabbits under chloroform and ether. Those in Fig. 3 are typical of moderate degrees of anæsthesia (corneal reflex just abolished). The slow waves have a characteristic frequency of 3–4 per sec. with brief waves at 25–40 per sec. superimposed. Simultaneous records of the electrocardiogram and of respiration show that the cortical rhythm is quite independent, although these extraneous rhythms are often of the same order. In very deep anæsthesia (Fig. 4), the 3–4 per sec. rhythm gives way to a slower beat at intervals of 1–2 sec. The superimposed brief waves are on the whole more regular, and their frequency usually lies in the region of 25–30 per sec.

Both slow and brief waves are due to the activity of the cortex. It is true that when a large earthed electrode is used they persist after the

cortex under the grid electrode has been killed by freezing with solid CO_2, but this is due to the dead tissue acting as a lead from neighbouring

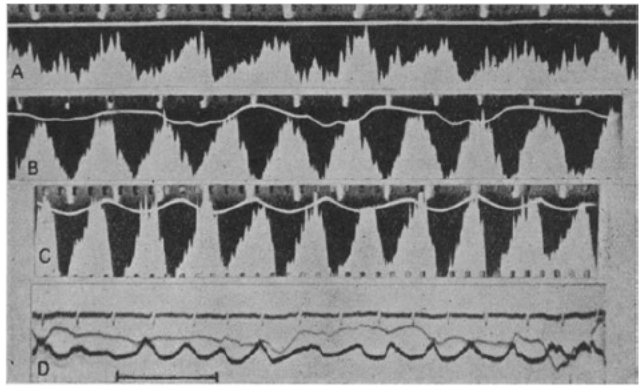

Fig. 3. Rhythmic waves from the rabbit's cortex in moderate C. and E. anæsthesia. Four different animals. Slow waves at 3–4 per sec. with brief waves superimposed. Respiration shown in B and C and electrocardiogram in D (upper line), not in phase with the slow waves. In D there are two records from different regions but the rhythm only appears in the lower. Time marker in A, B and C gives 1/4 sec. In D horizontal line = 1 sec.

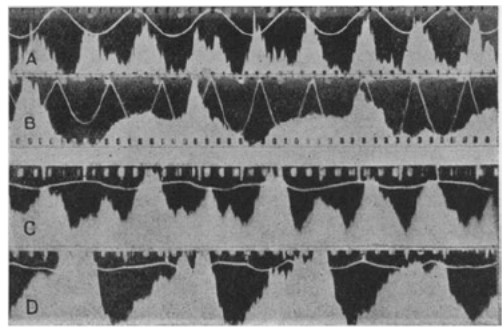

Fig. 4. Slower rhythm in deep C. and E. anæsthesia. A and B from one rabbit; A, moderate anæsthesia, waves 3·5 per sec.; B, deep, waves 1 per sec. C and D from another rabbit; C, moderate anæsthesia, waves 4 per sec.; D, deep, waves 1·3 per sec. Respiration not in phase.

regions, for with the balanced amplifier two electrodes on the killed area record little or nothing. Subcortical structures may possibly contribute to the effect under certain conditions, but we have no evidence as to this.

(*b*) *The injury response.* Another characteristic type of activity is that produced by injury to the cortex. It can be produced without fail, and it supplies important evidence as to the nature of the potential waves. If two electrodes rest at some distance apart on the cortex and a small cut is made in its surface near one of them, there is usually an outburst of brief potential waves in a regular series with a frequency somewhere between 90 and 35 per sec. A cut 1 mm. long and ½ mm. deep is often enough, but a puncture with a fine wire to the same depth is usually without effect. On the other hand if a wire, a pointed gelatin electrode, or a concentric needle system is gradually pushed through the superficial layers of the cortex there is nearly always an outburst of the regular waves when the point has penetrated to a depth of 1·5–3 mm.

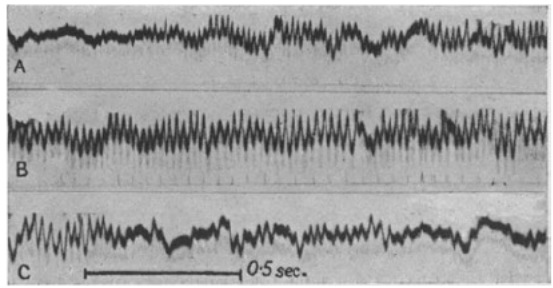

Fig. 5. Evolution of a typical "injury discharge." Rabbit under urethane. Puncture of cortex to 2–5 mm. depth by a fine silver wire forming the input electrode. A, beginning of discharge, frequency 64 per sec.; B, middle, frequency 44 per sec.; C, end, frequency about 40 per sec. during regular periods.

These injury discharges are clearly due to structures in the grey matter. In the rabbit's brain this has a thickness of about 2·5 mm., but the response of the white matter can be readily distinguished, for at a depth of about 3 mm. the response changes to a succession of small and very rapid oscillations like those given by a large nerve trunk pierced by a needle electrode. They are evidently due to impulse potentials in the axons, whereas the injury discharge is due to nerve cells and dendrites[1].

The response of the grey matter, whether to superficial or deep injury, shows a characteristic evolution as well as a characteristic frequency

[1] As there is no term which covers both the nerve cell and its dendrites but excludes the axon, we have spoken of the "cortical neurones" or "cortical nerve cells" as responsible for the potential waves. Some nervous constituent of the grey matter is responsible for them, cell bodies or dendrites or both.

range. The waves often begin a few seconds after the injury. They are small at first and can sometimes be seen as discrete, monophasic spikes with a duration of about 5–10σ. They become larger, and as they do so their outline broadens until the record becomes a sinusoidal oscillation. Usually the frequency reaches the maximum value (60–80 per sec.) at once and then falls gradually to a value in the region of 50 per sec. Occasionally the initial frequency is about 40–50 per sec. and the maximum is not reached for several seconds. The discharge may continue at about 50 per sec. for as long as half a minute, but eventually the frequency falls to 35–40 per sec., the waves begin to vary in size and the regularity ceases (Fig. 5). With more widespread damage the response usually appears after a shorter interval: the initial frequency may be as high as

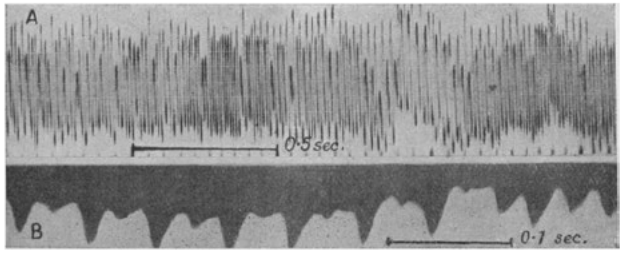

Fig. 6. A, beginning of injury discharge (rabbit, urethane). Waves alternately large and small, frequency 90 per sec. B, beginning of injury discharge (rabbit, urethane). Monophasic waves, frequency 40 per sec. (increased later).

100 per sec., and in the later stages occasional very large waves may occur, often diphasic. In many discharges there is a stage in which the successive waves are alternately large and small (Fig. 6), and sometimes the final break up of the rhythm is heralded by an alternating rhythm of this kind. Injury discharges have been recorded in twenty or more rabbits, and the frequency limits have shown no significant variation. The response to a superficial injury can be given a faster or slower rhythm by irrigating the surface of the cortex with hot or cold Ringer. The depth of anæsthesia has no obvious effect on the frequency, but in very deep C. and E. anæsthesia a greater injury may be necessary to produce the discharge. More severe injury seems to be needed in the cat than in the rabbit, though the discharge has the same frequency range and the same evolution (Fig. 7).

The characteristic evolution suggests that we are dealing with a synchronous pulsation in a number of units. Synchronous discharges

due to injury have been recorded in nerve [Adrian, 1930; Hoagland, 1933] and in muscle [Adrian and Gelfan, 1933], and in these structures, as in the cortex, the response consists of large regular oscillations which approach a sine curve but become small and less regular as soon as the frequency falls below a critical value. In the cortex it appears that the activity is at first confined to a few neurones and then gradually spreads to include more and more. This would account for the increase in size and the broader contour of the waves. Direct evidence of such a spread can often be obtained by recording simultaneously from two or three pairs of

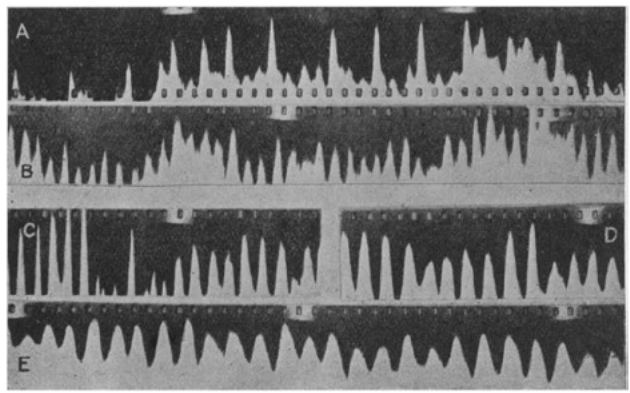

Fig. 7. Evolution of injury discharge in cat (C. and E. anæsthesia). Puncture of cortex by fine wire. A, large monophasic waves 30 per sec.; B, later, frequency 76 per sec.; C, later, frequency 72 per sec.; D, later, frequency 56 per sec.; E, later, frequency 48 per sec. Time marker gives 1/4 sec. intervals.

electrodes. Fig. 8 gives two examples of this. Further evidence of the synchronous pulsation comes from the fact that two electrodes placed very close together near the injured point often fail to show any sign of activity, whereas either of them in conjunction with a distant electrode shows large oscillations. Clearly no potential differences can be developed between two electrodes if both of them are on an area whose potential rises and falls synchronously at every point, but the oscillation will appear if one of the electrodes is removed to an inactive or less active region.

The units responsible for the injury discharge must have slower time relations than those of medullated nerve fibre. In a mammalian nerve trunk synchronous activity can occur over a frequency range of 600–120 per sec. In the cortex the range is about 100–35 per sec. Rhythms as high

as 200–300 per sec. have been observed occasionally with stimulation by a constant current, but 80–35 per sec. is the characteristic range. The form of the waves is another argument for slower time relations. The fully developed sinusoidal oscillation can only tell us that a number of units are pulsating together at a particular frequency, but there are often periods, sometimes at the beginning and sometimes towards the end of an outburst, when the waves are separated by distinct pauses (Figs. 6 B, 7 A).

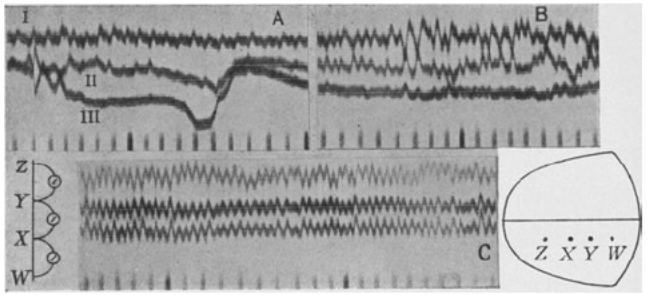

Fig. 8. Spread of injury discharge. Records from different points on the cortex (rabbit, urethane). A and B, successive stages in one experiment with four electrodes in line at intervals of 4, 3 and 3 mm. and three recording systems (cf. inset, full size). Injury at *Z* electrode: in A, record between *Z* and *Y* gives waves at 54 per sec. and that between *Y* and *X* gives half rhythm; in B, all three records give waves at about 45 per sec.; C, another injury giving waves at 50 per sec. between each pair of electrodes. Balanced amplifiers. Time marker gives 1/20 sec.

Since they still have a smooth contour and an over-all duration of about 5–10 σ it is unlikely that the response of each unit is much briefer than this.

The analysis of cortical potentials.

The nature of the injury response has been discussed at length because the waves which appear in it give a fair idea of the rapidity of the changes which can occur in excited nerve cells, and because they resemble very closely the brief oscillations which occur spontaneously. In the records of spontaneous activity already given (Figs. 2, 3, 4), the brief oscillations cannot be seen very clearly, but the size of the slow waves can be reduced by using smaller coupling condensers, and it is then possible to study the brief waves under higher amplification. Though they can always be observed if the amplification is great enough they are usually most prominent in moderately light C. and E. anæsthesia (Fig. 3 A). They are

certainly more variable than the waves of the injury discharge. Moderately regular waves at frequencies below 35 per sec. are often found, particularly in deep C. and E. anæsthesia, and there are occasional groups of frequencies as low as 10 per sec.; but regular oscillations at 35–50 per sec. are usually present in parts of the record, and it is often quite impossible to distinguish a regular train of spontaneous waves from the regular oscillation of an injury discharge (Fig. 9). It is true that no hard and fast line can be drawn between what we have called "brief" and "slow" waves, but there is no doubt that at the "brief" end of the scale the waves resemble those produced by injury to the cortex.

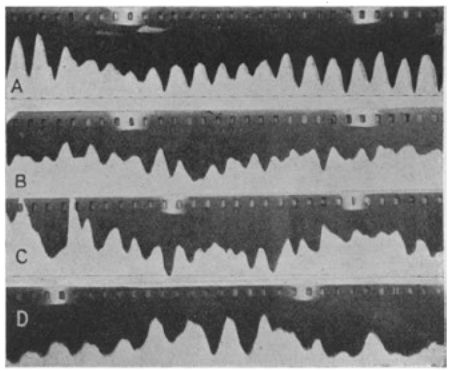

Fig. 9. Comparison of injury discharge and "brief waves" in spontaneous activity. Rabbit under C. and E. A, response to puncture of cortex: waves 48 per sec.; B, C and D, spontaneous activity in uninjured cortex with groups of brief waves; frequency between time signals, B 48 per sec., C 40 per sec. and D 36 per sec. (approximate). Time marker gives 1/4 sec.

Their likeness to the injury discharge shows that the brief waves represent the rapid pulsating activity of groups of nerve cells. Our problem, then, is to decide whether there is any other kind of activity. Are the slow waves merely summation effects compounded out of repeated brief pulsations in many neurones, or are some of the neurones capable of slow changes as well?

The fact that the brief waves in the injury discharge are initially monophasic has an important bearing on the question, for if the brief waves were invariably diphasic they could not summate to give a large, slow deflection. The waves which occur spontaneously are rarely separated by pauses distinct enough to show whether they are monophasic or

not, but at the beginning and sometimes at the end of an injury discharge there is no doubt of it. It might be argued that this is a natural consequence of injury, but the injury may be inflicted at some distance from the electrodes and the responses are still monophasic. Moreover, it would be natural to expect a monophasic potential change when a nerve cell becomes active, for one electrode may be regarded as leading from the point at which the activity originates and the other is a diffuse lead from

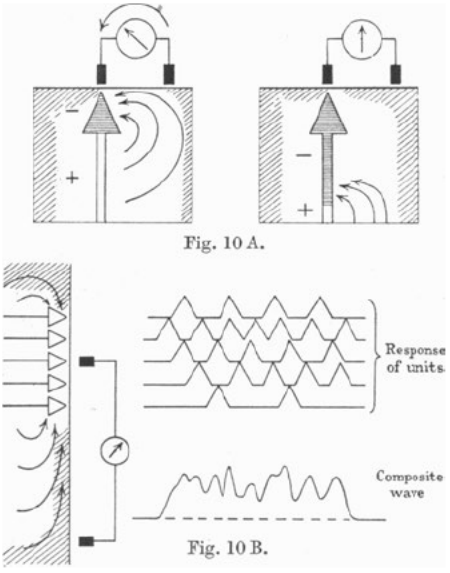

Fig. 10 A.

Fig. 10 B.

Fig. 10. A, potential gradients due to a wave of activity arising in a nerve cell and travelling down the axon, to show the production of monophasic waves: B, potential gradients due to repeated asynchronous activity in a group of nerve cells, to show the formation of the slow potential waves. For simplicity it is assumed that all the nerve cells will have the same effect on the recording system.

the whole of the axon (Fig. 10 A). Thus a series of rapid monophasic pulsations in a large number of neurones might give a composite potential wave in the way illustrated in Fig. 10 B. This would only occur if the pulsations were asynchronous, and it is noteworthy that in a synchronized injury discharge the slow waves disappear. This is perhaps the most direct evidence for the view that the slow waves are built up out of the same components as the injury discharge. The two types of activity are

mutually exclusive as they must be if one is due to asynchronous and the other to synchronous pulsations in the same neurones.

If the slow waves are in fact due to a summation of the brief waves they should be much more in evidence when the distance between the electrodes is large. If the distance could be made so small that the main effect came from a single neurone we should expect to find nothing but a succession of the brief waves, and the summation effect would become larger and larger as there were more and more neurones to contribute to it. On the other hand, if single neurones can produce slow as well as brief changes of potential these should be still evident when a very small electrode system is used.

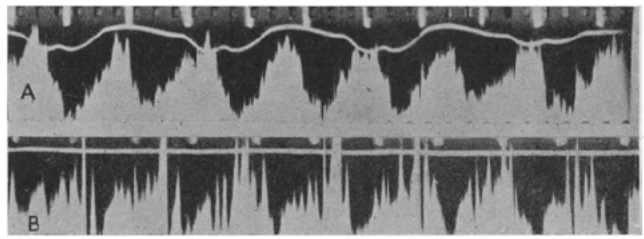

Fig. 11. Change in relative sizes of "slow" and "brief" waves with change in separation between electrodes, Rabbit under C. and E. A, input electrode on cortex, earthed electrode in cheek. B, concentric needle electrode system 1 mm. diam. on cortex. Recording system 10 times more sensitive. The brief waves are much larger, but the slow are if anything smaller than in A in spite of the increased sensitivity.

All our experiments agree in showing an increase in the size of the brief waves relative to the slow when a restricted electrode system is used. Thus Fig. 11 compares the rhythmic waves in C. and E. anæsthesia as recorded (*a*) with a diffuse earthed electrode and one lead from the cortex and (*b*) with a concentric needle system resting on the surface of the cortex. The amplification in (*b*) is greater than in (*a*), so that the reduction in the size of the slow waves is much greater than appears in the record. Again, Fig. 12 shows the typical urethane outbursts recorded with three pairs of pointed gelatin electrodes arranged as shown in Fig. 12 A. In the upper records the sensitivity is the same for all three pairs. There is no sign of the slow waves at the pair of electrodes which are only 1 mm. apart, though the brief waves are not very much smaller than at the more widely separated electrodes. In the lowest record the sensitivity has been greatly increased for the 1 mm. electrodes: the brief waves

are more prominent but the slow waves are still greater at the other electrodes.

A similar result is reached when we use electrodes at different distances from the surface of the cortex. Tönnies [1933] has already compared the effect of leading from the cortex, the dura and the unopened skull, and shown the greater prominence of brief waves in the cortical record. We have used the arrangement shown in Fig. 13. A pool of warm Ringer was formed on the surface of the cortex by building a wall of dental cement

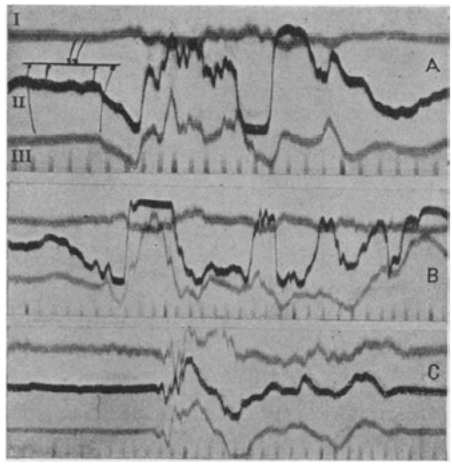

Fig. 12. Change in relative sizes of slow and brief waves with electrode separation. Rabbit under urethane. Balanced amplifiers. Electrodes arranged as shown in A; those to oscillograph I are 1 mm. apart, those to II 9 mm. and those to III 16 mm. In A and B all three oscillographs are equally sensitive. In C, No. 1 is made more sensitive (top record, 1 mm. between electrodes). Time marker gives 1/20 sec.

round the cut edges of the skull or by lifting up the scalp at each side. The electrodes were placed so that the points of one pair rested on the cortex with the others at various distances above it in the Ringer. The slow waves can be detected 6 mm. above the cortex, and in Fig. 13 B where the top pair of electrodes are 9 mm. apart and the bottom pair only 2 mm. there is no marked difference in their size, but in both records the brief waves appear only at the electrodes actually in contact with the cortex. Evidently the brief waves arise from structures very close to the surface electrodes, whereas the slow waves have a much wider or more distant origin. The diagram in Fig. 14 may make this clearer.

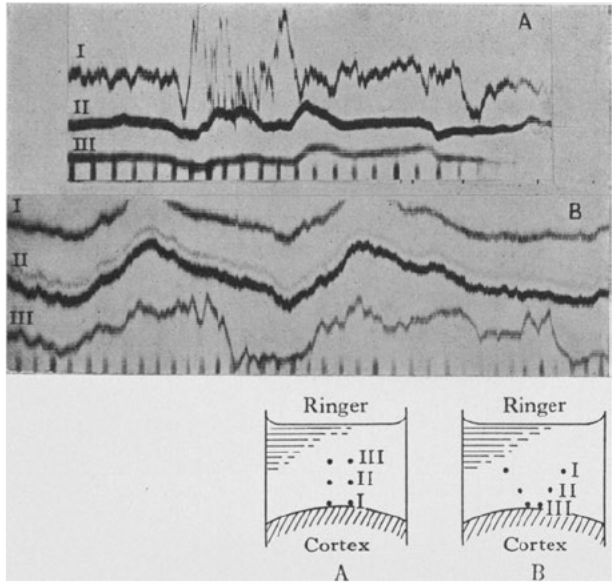

Fig. 13. Change in relative sizes of slow and brief waves with electrodes at different distances from surface of cortex. Pool of Ringer covering brain (rabbit, urethane). In A oscillograph I connected to electrodes 3 mm. apart on surface of cortex; II to electrodes 3 mm. apart and 3 mm. above surface; III ditto, 6 mm. above surface. I less sensitive —oscillographs adjusted to give approximately equal excursions.
In B, III is connected to surface electrodes 2 mm. apart; II to electrodes 4·5 mm. apart and 2 mm. above surface and I to electrodes 9 mm. apart and 5 mm. above surface. The very small oscillations in I and II are due to amplifier noise. Time marker gives 1/20 sec.

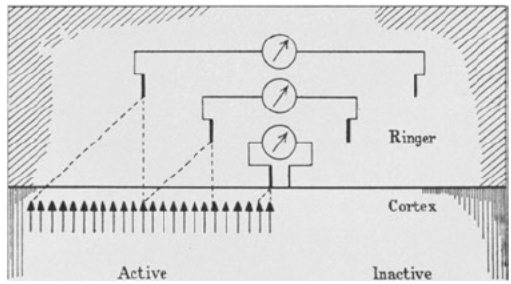

Fig. 14. Potential waves recorded by electrodes on the surface of the cortex and some distance above it (as in Fig. 13). The electrodes above the surface will record only the summated effect from a wide area. Those on the surface will be mainly affected by the neurones immediately under them.

Thus the composite nature of the slow waves is shown by our inability to localize their origin. They are greatest when a large mass of cortex intervenes between the two electrodes, but a detailed exploration of the mass, both in depth and on its surface, never reveals any region where the slowly developing potential gradients are greater than elsewhere. Their existence evidently depends on the activity of a large number of units, and the response of each unit bears no resemblance to them.

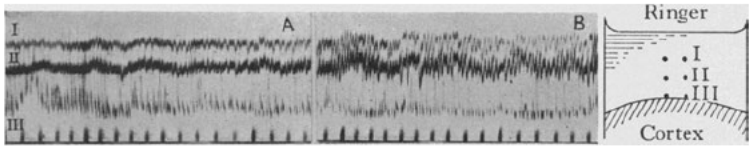

Fig. 15. Synchronized injury discharge from a large area appearing at electrodes 6 mm. above cortex. Rabbit, urethane. I, to electrodes 3 mm. apart and 6 mm. above surface (in Ringer); II, to electrodes 3 mm. apart and 3 mm. above surface; III, to electrodes 3 mm. apart and in contact with surface. In A the discharge is localized and has not much effect on the electrodes above the surface. In B it has spread and appears in all three recording systems. Time marker gives 1/20 sec.

Amplifier distortion.

As the foregoing experiments were all made with condenser-coupled amplifiers the slow waves will be distorted to some extent in the records. This does not affect the argument, but it is possible that the regions under the electrodes might sometimes change their potentials so slowly that nothing would appear in the record. To examine this possibility and generally to see how far our results are affected by amplifier distortion we have made some simultaneous records with a battery-coupled amplifier leading to one oscillograph and a condenser-coupled amplifier to another. With 1 mf. coupling condensers in the latter there is very little difference in the records, and in particular there is no indication of gradual (or sudden) shifting of the base line with the distortionless amplifier. Between the slow waves the potential returns to its original value just as it does with condenser coupling. Thus there is no evidence of very slow potential changes either in the cortex at large or, *a fortiori*, in the individual neurones.

Areas contributing to brief and slow waves. (a) The brief waves. Synchronous pulsation of neurones. The existence of the slow waves implies an asynchronous pulsation in the different parts of the area which contributes to the potential gradient, for if all the neurones were pulsating synchronously we should record a rapid oscillation instead of a slow rise or fall. The widespread synchronous pulsation caused by injury does in fact appear as such at electrodes as much as 6 mm. above the surface of the cortex (Fig. 15), but this distance is enough to smooth out all trace of the brief waves in the normal spontaneous activity. On the other hand, it is

unlikely that the brief waves are due to single nerve cells, for the magnitude of the potentials (which may be as much as 100 microvolts) and the sinusoidal wave form both suggest a synchronous pulsation in a group of cells.

We have attempted to find out how large an area contributes to each of the brief waves, for this will give some idea of the magnitude of the group which is pulsating together. As might be expected, the results show a considerable variation. In Fig. 16, for instance, there is an occasional agreement in some of the brief waves recorded at two pairs of electrodes 3 mm. from one another, but in a good deal of the record there is no agreement at all. This shows that there is no likelihood of an electrical spread from one pair of electrodes to the other and makes the occasional agreement more significant.

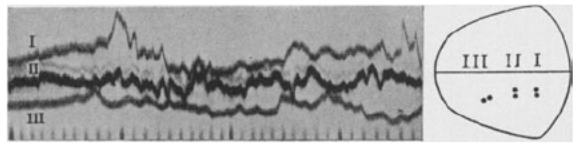

Fig. 16. Occasional agreement of brief waves at electrodes 3 mm. apart. Rabbit, C. and E. Pointed gelatin electrodes arranged in pairs, I, II and III (see diagram) with 1 mm. separation between the electrodes of each pair. I is 3 mm. from II and II is 4 mm. from III. In the first outburst the waves at I and II show some agreement, but there is very little in the later part of the record. No agreement at III. Time marker gives 1/20 sec.

From this and many similar records it appears that in the normal activity of the anæsthetized brain a synchronous pulsation does not cover an area of more than 3–4 mm. diam. and is often much more restricted. An area of 3 mm. diam. might be covered by the dendrites of a single nerve cell, but there is no reason to doubt that each brief wave is the product of a group rather than a unit. Synchronized action occurs, apart from gross injury, in different regions of the nervous system, *e.g.* in the motor centres of the cord, in the retina, the optic ganglion of *Dytiscus*, etc. It is most evident when there is an intense, uniform excitation of the whole region, and it might well occur in smaller areas if these were uniformly excited.

(*b*) *The slow waves. Rise and fall of activity in larger areas.* If the slow waves are due to summated brief waves from many neurones it follows that there must be a general agreement in the periods of rest and activity over considerable areas, although the neurones in them are not pulsating

in phase. The mere existence of a potential gradient shows, of course, that the activity is not uniform throughout the cortex. Indeed the size of the waves will be a measure of the gradient of activity along the line joining the electrodes, and by recording simultaneously at several points we can gain some idea of the distribution of activity from moment to moment.

It is found that the distribution is complex in light and moderate anæsthesia but simpler in deep anæsthesia; with light anæsthesia the waves at three pairs of electrodes 5 mm. or more apart show little or no

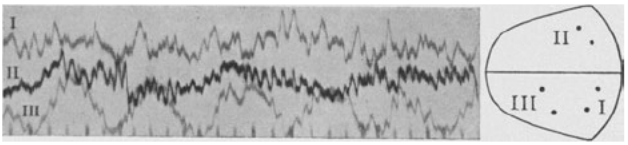

Fig. 17. Lack of agreement in activity at different points in moderate C. and E. anæsthesia. Thread electrodes, 4 mm. separation. Arranged as in diagram with 10 mm. between I and II and 5 mm. between II and III. Note continuous series of brief waves, and slow waves with regular 4 per sec. rhythm at electrodes III only. Time marker gives 1/20 sec.

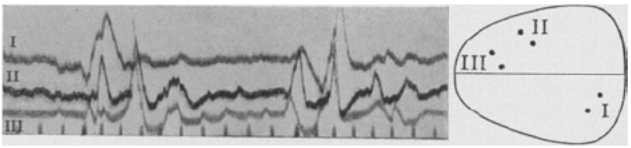

Fig. 18. Agreement of active periods in deep urethane anæsthesia (rabbit). Electrodes 3 mm separation arranged as in diagram. 15 mm. between I and II, 5 mm. between II and III. Time marker gives 1/10 sec.

correspondence (Fig. 17), but in deep anæsthesia they agree very closely. In the typical response under urethane the slow waves appear almost simultaneously at points 1 cm. or more apart (Fig. 18), and in deep C. and E. anæsthesia there is a regular cycle of rest and activity which is equally widespread (Fig. 19). This cycle has a period of 1–2 sec. and appears only when the anæsthesia is strong enough to threaten respiratory failure if its administration is prolonged. The regular waves at 3–4 per sec. which occur in lighter C. and E. anæsthesia are usually confined to one pair of electrodes, or appear with an independent rhythm at different points (Fig. 17).

Regular beating of this kind could scarcely occur except in a cortex undisturbed by external influences, and it is found that if sensory stimulation produces any effect at all that effect is to break up the rhythm. This is best seen in urethane anæsthesia light enough to allow a slight reflex contraction of the leg muscles when the foot is pinched. Illustrations of this are given in Fig. 20, and it will be seen that pinching the foot abolishes the slow rhythms and increases the prominence of the brief waves, which

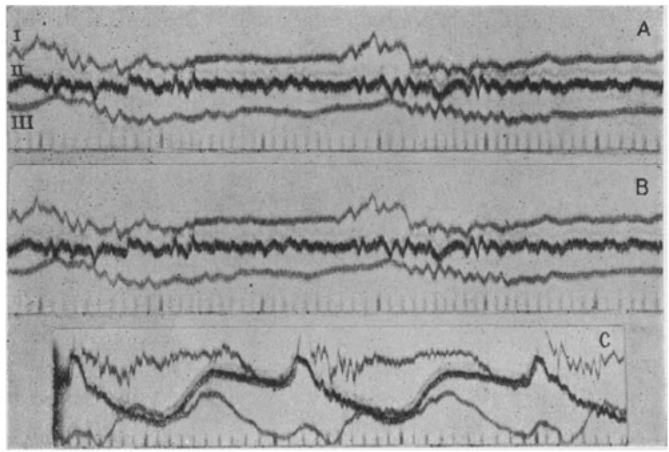

Fig. 19. Agreement of active periods in deep C. and E. anæsthesia (rabbit). A and B, pointed gelatin electrodes 1 mm. apart arranged in pairs as in Fig. 16 with 3 mm. between I and II and 4 mm. between II and III. Outbursts of brief waves at 1½ sec. intervals. C, another animal. Thread electrodes 4 mm. apart arranged in pairs as in Fig. 17. Outbursts at 1 sec. intervals. Note the presence of slow waves in C (electrodes 4 mm. apart) and their absence in A and B (electrodes 1 mm. apart). Time marker gives 1/20 sec.

now appear in a continuous outburst. In some records the slow waves have returned at a higher frequency towards the end of the stimulation.

It will be clear from Figs. 17–19 that in deep anæsthesia periodic waves of activity may sweep over the whole cortex (or rather over its dorsal surface), and that in lighter anæsthesia one or more regions may beat rhythmically at 3–4 per sec. Waves of the same kind occur in the cat as well as in the rabbit, though not with such uniformity. They are not in phase with respiration or the heart beat and are not appreciably altered by changing the blood-pressure or the composition of the air breathed by the animal. They may depend on subcortical mechanisms, but all that

can be said at the moment is that they show that the respiratory centre is not the only region of the brain which has a tendency to slow, rhythmic beating. The rhythmic movements of narcosis progression described by Graham Brown [1914] are probably due to beats of the same kind. Indeed, Graham Brown's suggestion of inherent rhythms modified by afferent control seems to be well illustrated by records such as those in Fig. 20.

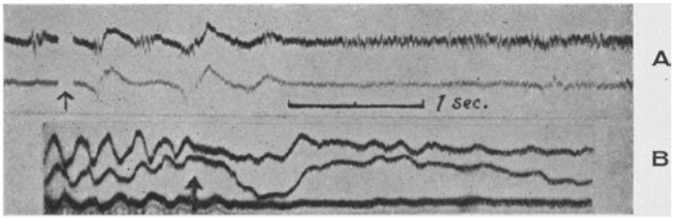

Fig. 20. Rhythmic outbursts cease on sensory stimulation in light anæsthesia. Both records are made with earthed amplifiers, not with the balanced type. The agreement in the waves may be due to the common earth leads. A, rabbit, urethane. Pinching foot at arrow. The slow waves give place to a continuous series of rapid oscillations. B, another animal. Pinching foot at arrow.

Transitional waves. (*a*) *Due to injury.* In the spontaneous activity of the anæsthetized brain there is no evidence of synchronous pulsation spreading over areas of more than 4 mm. diam. In the injury discharges much larger areas may be involved and there is often a progressive increase as the discharge proceeds. Thus the ease with which the pulsation of one neurone spreads to another can vary with the condition of the cortex. In certain conditions the spread takes place with abnormal ease, and a single pulsation may travel like a ripple over the surface of the brain, giving rise to a characteristic potential change.

This type of activity is often seen as a sequel to an intense injury discharge and it is invariably found with certain convulsant drugs. It leads to the production of very large potential waves, often with a smooth contour, representing a transition between the slow and the brief type.

The simplest case to take is that produced by injury. As a rule the rapid pulsation ceases by becoming irregular after the frequency has fallen below 35 per sec.; in some records, however, very large waves appear from time to time during the discharge and ultimately these become its most prominent feature. They may occur singly at intervals of

½ sec. or so, or in groups of three or four. Their potentials may be as great as 5 millivolts and they appear over a wide area. Examples of them are given in Fig. 21. The waves usually begin as simple monophasic spikes differing only in size from the other waves in the series (Fig. 21 C and D), but ultimately they may become di- or triphasic, or may present multiple crests and troughs as in Fig. 21 B. This change in appearance seems to be

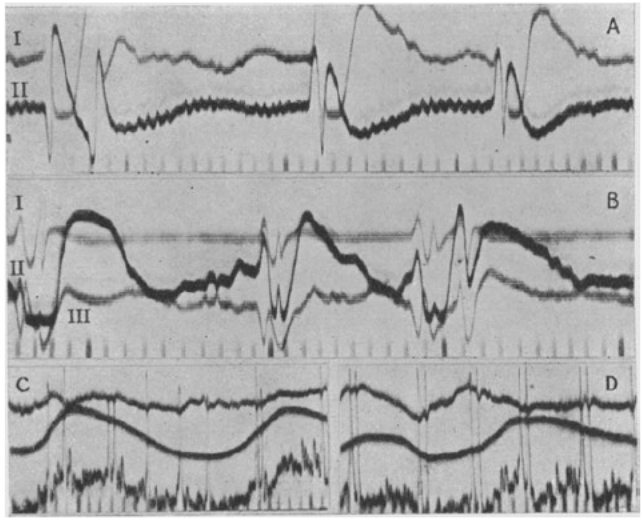

Fig. 21. "Transitional" waves due to injury. A, rabbit, urethane. Earthed amplifiers. Later stage of injury discharge with three large waves and small oscillations at 40 per sec. B, rabbit, urethane. Balanced amplifiers. Electrodes arranged as in Fig. 12. I 1 mm. apart, II 9 mm. and III 16 mm. Sequel to prolonged injury discharge. Note absence of slow waves at I. C and D, rabbit, urethane. Earthed amplifiers. Lowest record shows development of large transitional waves (monophasic) occurring singly at first and then in couples. Time marker gives 1/20 sec.

due to the waves or groups of waves spreading at a finite rate through the cortex so that the region of maximum negativity lies now under one electrode and now under the other. But the conduction takes place without the waves losing their individuality and an almost identical pattern of activity may appear at points 10 mm. or more apart.

The development of these large waves out of the brief pulsations of the injury discharge shows how a broader contour and a diphasic form may result from a pulsation which spreads over the cortex instead of being

confined to a small area. In the discharges produced by convulsant drugs we find the same kind of conducted pulsation and can sometimes follow the change from the transitional type into the usual complex of slow and brief waves.

(*b*) *Convulsant drugs, thujone.* Strychnine produces widespread synchronous activity in the nervous system and might therefore be expected to give a cortical discharge made up of widespread pulsations. Fischer [1933 *a*], Bartley [1933] and others have recorded the cortical response, and their records show a succession of waves of very large potential repeated as rapidly as 25 per sec., or occurring singly or in groups at lower frequencies. At the higher frequencies they have the simple contour of the waves in an injury discharge and at lower frequencies they resemble the large "transitional" injury waves shown above. Fischer has used other convulsant drugs, picrotoxin, caffeine, etc., with the same results. In our own experiments with convulsants we have generally used thujone, the active principle of wormwood oil. In the unanæsthetized animal thujone produces convulsions which are said to resemble those of epilepsy. In an animal deeply anæsthetized with urethane there is usually no motor effect, but there is an obvious counterpart of the convulsion in the potential record from the cortex.

We have used an emulsion of thujone in olive oil and gum arabic solution injected into the femoral vein. A dose of 0·5 c.c. of a 1 in 10 emulsion is enough to produce the typical cortical discharge in a rabbit under urethane. As a rule the first sign of the effect is that the slow urethane waves disappear: then after an interval of apparent inactivity there is a more rapid series of slow waves, each with a small group of brief waves superimposed. Very soon, instead of a group of brief waves on a slow deflection, we find a sudden large oscillation, wider and more complex in form than that of the usual brief waves, but no longer clearly divisible into fast and slow components. A series of records showing the evolution of a thujone discharge is given in Fig. 22. To avoid confusion only one oscillograph tracing is shown in the figure, although three simultaneous tracings were made from different points. It will be seen that the discharge works up into a series of large diphasic waves of simple contour; the wave form changes from time to time and ultimately the discharge stops suddenly and completely.

In this particular record the frequency of the large waves is never greater than 10 per sec., but it may reach 20 per sec. or more with larger doses, and the large beats are sometimes preceded or followed by smaller 50 per sec. oscillations.

The factors underlying the wave form and the changes it undergoes can be studied by simultaneous recording at different points. At the height of the discharge the whole surface of the cortex pulsates with the same rhythm though occasionally a beat is missed at one pair of elec-

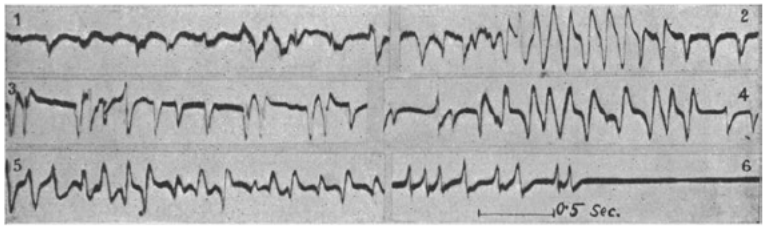

Fig. 22. Evolution of discharge due to thujone in a rabbit under urethane. Portions of a continuous record, intervals of 2–3 sec. between successive strips. Pointed gelatin electrodes 1 mm. apart on surface of cortex. Three simultaneous records were made from different points but the tracings of oscillographs I and III have been painted over to show II more clearly. The largest deflections represent potential changes of about 5 mv.

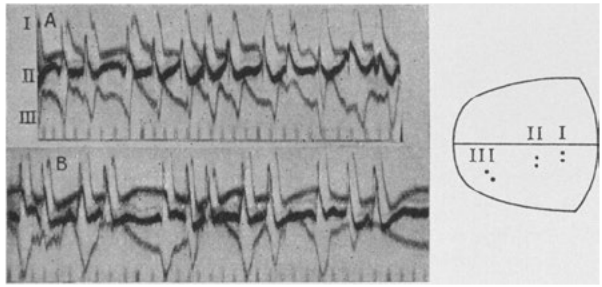

Fig. 23. Record of thujone discharge from three points on the cortex, showing agreement of waves. A, near the beginning of the discharge, waves 10 per sec. One wave is missed at III (8 mm. from II), but otherwise there is complete agreement. B, later; I and II still agree, but more waves are missed at III. Gelatin electrodes 1 mm. apart, 4 mm. between I and II, 8 mm. between II and III. Time marker gives 1/20 sec.

trodes (Fig. 23). More information can be gained, not from three pairs of electrodes a long distance from one another, but from four electrodes in line (*W*, *X*, *Y*, *Z*, Fig. 24) with the three recording systems between adjacent electrodes. With such an arrangement we can map out the potential distribution along the line and see how it changes from moment

to moment. Two characteristic records from the same discharge are given in Fig. 24. In Fig. 24 A the waves have a broad contour, they are diphasic and the maximum negativity of *W* relative to *X* occurs about 0·025 sec.

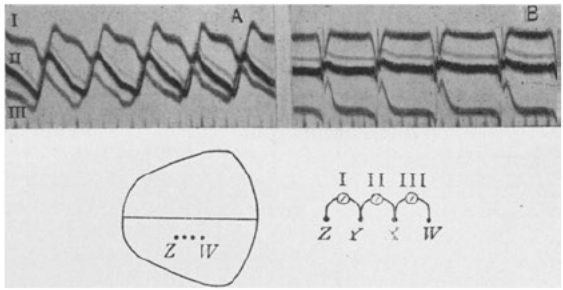

Fig. 24. Thujone waves recorded at four electrodes in line, 1 mm. apart. Two strips from a continuous record. Rabbit, urethane (deep). 0·5 c.c. of 1 in 10 thujone emulsion into femoral vein. Electrodes etc. arranged as in diagram, at *W*, *X*, *Y*, *Z*. Negativity of *W* relative to *X*, *X* to *Y* and *Y* to *Z* gives a downward deflection. In A the wave is simple and travels slowly from *W* to *Z*, in B it is complex and travels much more rapidly, see analysis in Fig. 27. Time marker gives 1/20 sec.

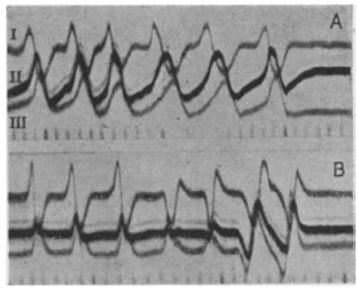

Fig. 25. Thujone waves with four electrodes in line 1 mm. apart, showing progressive changes in rate and direction. Electrodes arranged as in Fig. 24. In A the wave starts from *Z*, and successive waves spread more and more slowly until the discharge ceases. In B the wave starts from *Z* at the beginning of the record and has come to start from *W* by the end of it. Time marker gives 1/20 sec.

before that between *X* and *Y*, and 0·05 sec. before the maximum between *Y* and *Z*. In Fig. 24 B the waves are sharper and more complex and they occur almost together in each recording system. It follows that in the upper record a single pulse of activity begins at *W* and spreads relatively slowly to *Z*, whereas in the lower there are several pulses and the rate of

spread is very much more rapid. In Fig. 24 the wave form remains very nearly constant throughout the record, but there is often a gradual change from moment to moment. Thus in Fig. 25 A as the frequency of the beat declines the activity spreads more and more slowly along the line (starting in this case from the *Z* end). In Fig. 25 B there is a complete reversal during the seven waves in the record: the activity starts from the *Z* end in the first beat, in the middle of the series it appears first in the region *X Y* and by the end it has come to spread down the line from *W* to *Z*. The change in velocity and direction often occurs quite suddenly, as in Fig. 26 A, where a single beat starts from the *W* end, and Fig. 26 B, where there are several abrupt changes in the rate of spread. The rhythm may be disturbed by these changes, but it is never disturbed enough to suggest that an entirely

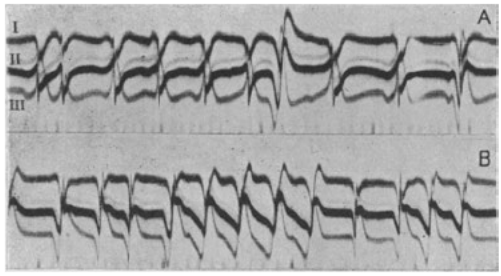

Fig. 26. Sudden changes in direction and rate of spread. Electrodes arranged as in Fig. 24. In A a single beat starts from *Z*. In B the rate of spread changes abruptly from time to time. Time marker gives 1/20 sec.

independent source of activity has taken charge. The potential gradients between the four electrodes at different moments are represented, for a few typical beats, in Fig. 27. They show that the beat sometimes involves a double pulsation in part of its course and that it travels at a variable rate, sometimes in one direction and sometimes in another. The change in rate may well be due simply to the change in the direction of the wave, for if it travels in a direction parallel to the line joining the electrodes the rate of conduction will appear much slower than if the direction is nearly at right angles. The rate in Fig. 24 A is of the order of 10 cm. per sec., but the uncertainty as to the direction of travel makes it impossible to say how far this applies to all the beats.

The sudden or gradual shifts in direction are not surprising if we assume that the cortex behaves under thujone like a mass of cells as closely linked as are the muscle fibres of the heart. Spontaneous beats

may arise now from one point and now from another, or the origin may shift progressively without affecting the rhythm. Similar changes often take place in the spontaneous beats produced in skeletal muscle fibre bathed in NaCl solution. A record illustrating this is given in Fig. 28, and it will be seen that the origin of the beat shifts gradually during the discharge so that the action potentials come to be reversed in phase, as in the

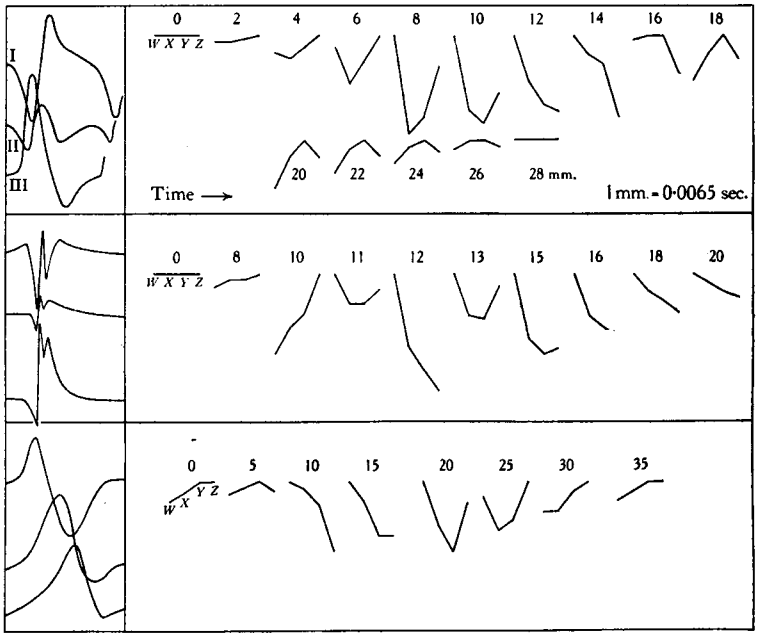

Fig. 27. Relative potentials at the four electrodes, *W*, *X*, *Y* and *Z*, at different periods during the passage of a wave. Tracings of the oscillograph record are given on the left. The potential distribution is given at various intervals after the beginning of the wave.

cortical record in Fig. 25 B. It is possible that the cortical beats arise as the result of impulses coming from subcortical structures, though there is evidence to show that in certain forms of experimental epilepsy the fit spreads by conduction in the cortex. There is no reason to suppose, however, that the thujone waves will always spread uniformly. Some areas may be more fatigued than others, and these may form occasional islands of inactivity round which one wave must travel though the next may find them responsive again.

As the effect of the drug wears off there is a gradual return to the usual complex of brief and slow waves typical of urethane—as though the syncytium were breaking up again into smaller and smaller groups.

For the present argument the chief interest of the thujone discharge is that it gives no evidence of distinct brief and slow components in the cortical response. The diphasic form of the waves is obviously due, as in a nerve or muscle fibre, to the passage of the active region from one electrode to the next, and the monophasic potentials in each neurone may well be quite as brief as they are in the typical injury discharge.

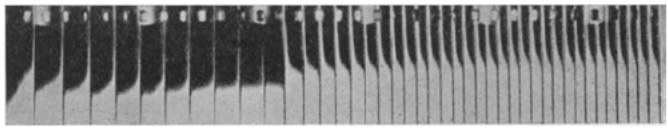

Fig. 28. Record of action potentials in a muscle fibre of the frog's sartorius immersed in NaCl solution, showing progressive shift in the origin of the waves (compare with Fig. 25 B). Muscle in bath divided by a slot, leads taken from the fluid on either side. There is a gradual reversal of the waves as the origin shifts from one side of the slot to the other. Maximum frequency 44 per sec. Time marker gives 1/4 sec. Battery-coupled amplifier.

Discussion.

The main outcome of these experiments has been to satisfy us that the potential waves in the cortex are all built up out of relatively brief pulsations in the individual neurones, that the slow potential changes are summation effects and have no counterpart in the response of the units. We set out with a bias towards the opposite conclusion and for a time our results seemed to support it. The turning point came when we began to use the balanced amplifier system for independent recording from several points. This gave conclusive evidence that the slow waves needed large areas for their development and showed how the earlier results were vitiated by the use of diffuse earthed leads.

Although the time relations found in the injury discharge have been surprisingly constant in all our experiments we can scarcely conclude that the response of all the cortical neurones is a series of brief waves of identical duration. The constant form of the action potential in a nerve trunk seemed to indicate uniformity in the constituent fibres until Gasser and Erlanger [1927] showed how widely the units might differ. There may be as great or greater differences in the units of the cortex, though, as in nerve, the major part of the electric effect is probably due to a more or

less homogeneous group. Again, we cannot be sure that the pulsations of a given cortical unit do not vary from moment to moment, *e.g.* with fatigue. All that can be said is that the response is probably a simple monophasic spike which occupies not much more than 0·1 sec. and not much less than 0·01 sec. We cannot say that the responses do not vary in size, but all the variations we have found could be explained as due to changes in the number of units in action, and it can often be shown that an exceptionally large wave is in fact the product of a greater number of neurones.

Thus the cortical waves seem to be built up out of relatively simple components, rhythmic pulsations in the nerve cells varying in frequency like the impulses in a nerve fibre but not necessarily varying in any other way. The complexity of the cortical response is due to the complexity of the structure which gives rise to it, not to a more variable type of activity in the units. This means that we must abandon the hope that the slow potential changes in the cortex might be an index of slow change of polarization in the nerve cells and so an index of the rise and fall of the excitatory state which preludes activity. Slow changes may occur, indeed the work of Gasser and Graham [1933] on the after-potentials in the spinal cord and of Eccles [1934] on the cervical sympathetic ganglia makes it probable that they do occur—but in the cortex they must be so much smaller than the "spike" potentials that in the normal spontaneous activity they cannot be detected.

This leads at once to the further question as to the origin of slow potential changes in other parts of the nervous system. The slow respiratory waves in the brain stem of the goldfish [Adrian and Buytendijk, 1931], in the thoracic and optic ganglia of *Dytiscus* [Adrian, 1932] and in the retina have all been considered as evidence favouring the idea of slow potential changes in individual nerve cells. Their smooth contour was the main reason for supposing that they were not summation effects, but the smooth contour of many of the slow waves in the cortex makes it very doubtful if the argument can be sustained. The fact that the potential wave of the nerve cell is much less abrupt than that of the axon is probably a sufficient explanation even for the slow changes in insect ganglia.

Effects of anæsthesia.

It must be admitted that all our observations on the cortex in animals have been made under general anæsthesia. There was no lack of electrical activity, but in the unanæsthetized brain it might turn out to be an

activity of an entirely different order. As regards the individual neurones, however, there is no evidence to suggest any radical difference. Fischer, Kornmüller and Tönnies have worked on animals immobilized by curare, and their records have shown the same mixture of slow and brief waves, and Bartley and Bishop [1933] state that the only definite change as anæsthesia develops is a slight reduction in the magnitude of the potential waves. With light as opposed to deep anæsthesia we have found a more continuous and more irregular succession of waves with a more complete lack of correspondence in the waves at different points. In this condition the cortex may be accessible in a slight degree to afferent impulses, for stimulation may change the nature of the response for a time (*vide* Fig. 20). It is probable, then, that in the lightly anæsthetized and still more in the unanæsthetized brain afferent impulses will always break up any tendency to uniform activity. With deeper anæsthesia there is less to interfere with the spontaneous activity of the nerve cells, and we find more regular waves and greater uniformity in different regions.

With C. and E. there is a gradual diminution in the size of the waves as the anæsthesia deepens, but the final disappearance of activity occurs abruptly within a few seconds of the failure of respiration (either before or after) and coincides with the blanching of the cortex. It seems to be due not so much to the direct action of the anæsthetic on the cortex as to the sudden fall of blood-pressure. As soon as the circulation is restored (by artificial respiration and massage of the chest) the potential waves reappear, often as large rhythmical outbursts with intervals of complete rest, returning later to the more usual type of response. Failure of the oxygen supply is thus the most important factor in the failure of the electrical activity. The dependence on oxygen supply can also be shown by giving a few breaths of nitrogen or by compressing the vertebral arteries (the carotids having been ligatured previously).

It is remarkable to find so much electrical activity in the anæsthetized cortex and still more to find that it is not so much a random activity of independent units as a series of disturbances spreading over large areas. With thujone, for instance, the whole cortex may pulsate with the same rhythm, and waves seem to be conducted freely in all directions: this may occur in an animal in urethane anæsthesia deep enough to prevent any movement during the thujone discharge and to prevent any sensory stimulation from reaching the cortex and modifying its response. All this supports the suggestion made by Bartley and Bishop [1933] that the chief effect of the anæsthetic is on the afferent and efferent pathways

rather than on the cortex itself, but more evidence is needed before any certain conclusion can be reached. It is at least clear that the same physical laws will govern the potential distribution in the unanæsthetized brain, so that there will be the same opportunity for summation effects from large areas.

To sum up—our evidence points to the conclusion that the electrical activity of the cortex is due to events of the same character as those taking place in active nerve or muscle fibre, *i.e.* to successions of brief action potentials repeated rhythmically with a frequency which can vary within wide limits. Arguing from nerve and muscle fibre we may expect that there is little or no gradation in the electrical response of each unit apart from the change in frequency. The evidence on this point is scanty, and all that can be said is that an increase in the size of the brief waves is usually found to involve an increase in the area contributing to the wave. But this, in itself, is an important distinction between the behaviour of the cortical units and those of a nerve trunk. In the latter the units, if undamaged, are completely independent. In the cortex they pulsate in groups small or large. With intense excitation rapid synchronous pulsations may extend over an area of 5 mm. or more diameter, and from time to time a single beat may travel through the entire cortex. In fact with a focus of intense excitation (*e.g.* from injury) or under the action of a convulsant drug the mass of cortical neurones behaves more like the linked fibres of the heart muscle than the independent fibres of a skeletal muscle or a nerve. In the unanæsthetized brain the constant arrival of afferent impulses at different points must break up the tendency to synchronous action. It must be remembered, however, that we are dealing throughout with brains which are very small compared to that of man, and that the largest areas of synchronous action which we have found would occupy only a very small fraction of the human cortex.

Summary.

1. Potential changes recorded from the cortex of the anæsthetized rabbit are roughly divisible into large slow waves and smaller brief waves superimposed on the large. We have tried to find out what kind of electrical activity takes place in the individual neurones of the cortex to give rise to these waves.

2. In the light anæsthesia the waves occur irregularly, but in deeper anæsthesia there is much greater regularity, the slow waves occurring at intervals of 1–2 sec. in deep C. and E. or urethane and at 3–4 per sec. in

moderate C. and E. anæsthesia. In lighter anæsthesia sensory stimulation often abolishes the rhythmic beat and substitutes a continuous train of brief waves.

3. Injury to the cortex produces a characteristic discharge of brief waves in a rapid sinusoidal oscillation. The frequency falls gradually from a maximum of 100–60 per sec. to a minimum of 40–35 per sec. Below this the discharge becomes irregular. By recording simultaneously with three pairs of electrodes it can be shown that the potential changes in the injury discharge often occur over an area 5 mm. or more in diameter. They are probably due to synchronous pulsation in a large number of cortical units.

4. The injury discharge shows that the cortical neurones may develop a pulsating activity, like that of a nerve or muscle fibre. The brief waves in the response of the uninjured cortex are due to an activity of the same kind. The problem is to decide whether the slow waves represent a different kind of activity, or whether they are merely due to a summation of the brief waves.

5. If the slow waves are summation effects they should be present when the electrodes are widely separated, but absent or very small when they are close together. It is found that they are only present when the electrodes are arranged so that large areas can contribute to the potential changes. With restricted electrodes only the brief waves appear. It is concluded that the response of the individual neurone is a brief pulsation and not a slow change.

6. In the anæsthetized, but uninjured, brain the neurones tend to pulsate in small groups covering an area not more than 3–4 mm. diam. Periodic waves of activity may spread over the whole cortex in deep anæsthesia, but the neurones which take part in it still react in small groups which pulsate out of phase with one another.

7. After severe injury or under the action of a convulsant drug (thujone) a pulsation may spread widely through the cortex. The potential waves are then of "transitional" type and are no longer divisible into slow and brief components. An analysis of the waves in the thujone discharge shows that the pulsations often change their direction of travel. The cortex behaves as a freely conducting mass with beats starting now from one focus and now from another. Each beat probably represents a single brief pulsation, or rapid series of pulsations, in the individual neurones.

8. The effects of anæsthetics are discussed. It is suggested that their main effect is on the afferent and efferent pathways rather than on the

cortical neurones themselves, and that these are not likely to show any different type of electrical activity in the unanæsthetized brain.

9. It is concluded that the activity of the cortical neurones consists of a series of brief pulsations which can vary in frequency but probably not in size. Their time relations are not as rapid as those of the action potential in a nerve fibre. Except with intense excitation the frequency of the discharge does not rise much higher than 50 per sec.

It has not been possible to detect very slow changes of potential apart from summation effects. Gradual changes of polarization may occur, but the electrical effects must be much smaller than those due to the brief action potentials. Many of the slow potential changes in other preparations of the central nervous system may be due, like the slow waves in the cortex, to a summation of the brief responses of individual neurones.

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